UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE EFFECTS OF 12 WEEKS OF WALKING WITH AND WITHOUT BLOOD FLOW REDUCTION ON BONE TURNOVER MARKERS IN COLLEGE-AGED WOMEN

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By
KAELIN YOUNG
Norman, Oklahoma
2012

Downloaded from SHAREOK. Persistent Handle URL: https://hdl.handle.net/11244/319508

THE EFFECTS OF 12 WEEKS OF WALKING WITH AND WITHOUT BLOOD FLOW REDUCTION ON BONE TURNOVER MARKERS IN COLLEGE-AGED WOMEN

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

BY

_____
Dr. Debra Bemben, Chair

_____
Dr. Michael Bemben

_____
Dr. Travis Beck

_____
Dr. Allen Knehans

_____
Dr. Ari Berkowitz

© Copyright by KAELIN YOUNG 2012
All Rights Reserved.

ACKNOWLEDGEMENTS

This project would in no way have been possible without the help and contribution of many others. I would like to thank the women who participated as research subjects in this project. The differing personalities made each exercise session a new adventure. I owe a great debt to my graduate student peers who chose to spend their valuable time helping with exercise testing and training. Christopher Poole (AKA: CP), Daeyeol Kim (AKA: DK), Jeremy Loenneke (AKA: J Lo), Christopher Fahs (AKA: Fahs Daddy), Xin Ye (AKA: Big Xin), and Steve Ferguson (AKA: Stevo), nick-named with passion and affectionately known as the "A-Team". A special thanks to my lab-mates Pragya Sharma-Ghimire and Zhaojing Chen for their assistance during blood handling and processing. Thank you all so much!

I would also like to thank each of my committee members for their time and comments throughout this process. Dr. Berkowitz, I enjoyed your class and learned a great deal about the neural control of movement. I appreciate you taking the time to sit on my committee as the outside member. Dr. Knehans, I appreciate your knowledge and comments on the nutritional side of our field. I do have to say that entering and analyzing food logs was my least favorite part of this project. Nonetheless, thank you for your time and commitment for being a part of my committee. Dr. Beck, I have learned a great deal from you and have enjoyed our late night conversations about resistance training, farming, and statistical analyses. I can only hope you will still respond to my emails after I am gone! Truly, thank you for all of your help and insight over the last four years, it will never be forgotten. Dr. Mike, I cannot thank you enough

for your help and guidance over the last four years. As busy as you are with the day to day, I felt like you always made time to listen to any concerns or questions and lend some great thoughts and advice when needed. Furthermore, a huge thank you for allowing me to use your laboratory for testing and training during the study. Finally, none of this would be possible without Dr. Debra Bemben. Dr. Deb, you went out on a limb and chose to take me in to your lab. Because of you, I have been given the opportunity to continue doing what I love for a career, and for that, I am forever grateful to you. In the last four years, I have learned a tremendous amount from you. Not just lab techniques or knowledge pertaining to our field, but to take the time to do everything the right way and not to cut corners. Thank you so much for putting up with me, as I am sure you lost a few hours of sleep along the way. If it makes you feel any better, I have lost a significant amount of hair over the last four years. But in the end, the time I was able to spend with you and in your lab was invaluable to me.

Lastly, I have to thank my family and friends who supported me over the last 10 years. A special thank you to John "Jr." Krawiecki and Billy Bryant for their encouragement and support throughout life and during this scholastic adventure. To my Mother and Father, my accomplishments are a direct result of the traditions and values that you raised me with. People make the difference in life, not things. I love you both.

TABLE OF CONTENTS

CHAPTER I INTRODUCTION 1
Purpose 5
Research Questions 6
Hypotheses 6
Subquestions 6
Subhypotheses 6
Significance of the Study 7
Assumptions 8
Delimitations 8
Limitations 8
Operational Definitions 9
CHAPTER II REVIEW OF LITERATURE 13
Introduction 13
Bone Physiology and Structure 14
Mechanical Loading 19
Biochemical Markers of Bone Turnover 23
Exercise Interventions and BTM's in Premenopausal Women 26
Exercise Interventions and BMD in Premenopausal Women 29
Blood Flow Restriction Training 33
Summary 38
CHAPTER III METHODS 39
Subjects 39
Inclusion Criteria 40
Exclusion Criteria 40
Research Design 41
Exercise Protocol 43
Questionnaires 44
Body Composition/Bone Mineral Density 44
Strength Testing 47
Aerobic Capacity 48
Blood Sampling 49
Bone Turnover Marker Assays 49

Data Analyses 50
CHAPTER IV RESULTS AND DISCUSSION 52
Subject Characteristics 52
Areal Bone Mineral Density 53
Serum Markers of Bone Turnover 54
Body Composition 56
Muscle Strength 59
Aerobic Capacity 62
Bone Characteristics of the Tibia 63
Dietary Analysis 68
DISCUSSION 71
CHAPTER V CONCLUSIONS 84
Research Hypothesis 1 84
Subhypothesis 1 85
Subhypothesis 2 85
Subhypothesis 3 85
Subhypothesis 4 85
Significance of the Study 86
Future Research 86
REFERENCES 88
APPENDICES 100

LIST OF TABLES

Table 1. Baseline Subject Characteristics 53
Table 2. aBMD of the Total Body, Hip, and Spine Before and After Training…… 54
Table 3. Serum Markers of Bone Turnover Before and After Training 55
Table 4. Measures of Body Composition Before and After Training 57
Table 5. Muscle Strength Before and After Training 60
Table 6. VO_{2peak} Before and After Training 62
Table 7. Bone Characteristics at the 4% Tibia Site before and After Training 64
Table 8. Bone Characteristics at the 38% Tibia Site before and After Training 66
Table 9. Bone Characteristics at the 66% Tibia Site before and After Training 67
Table 10. Percent Changes in Bone Characteristics at the 4%, 38%, and 66% Tibia Site 68
Table 11. Average Daily Nutrient Intake During Weeks 1 and 12 69

LIST OF FIGURES

Figure 1. Percent Change in Bone Turnover Markers After Training 56
Figure 2. Change in Body Weight after 12 Weeks of Training..... 58
Figure 3. Percent Change in Body Composition Variables from Baseline 59
Figure 4. Change in 1RM Knee Extension Strength After Training 61
Figure 5. 1RM Knee Extension and Flexion Strength percent Change From Baseline .. 62
Figure 6. VO_{2peak} Percent Change from Baseline..... 63
Figure 7. Change in Daily Total Caloric Intake from Weeks 1 to 12..... 70

ABSTRACT

Walking while reducing blood flow to and from the working muscles has been shown to result in strength gains and increased muscle cross-sectional area (MCSA) in young men and older adults. However, little is known about the effects of this type of novel exercise on bone metabolism and bone health. **PURPOSE**: To determine the effects of 12 weeks of low-intensity treadmill walking with and without blood flow restriction (BFR) on serum markers of bone metabolism in college-aged women. Secondary objectives were to examine changes in thigh and calf MCSA, muscle strength, aerobic capacity, and bone characteristics of the tibia following the intervention. **METHODS**: Thirty-one young women, aged 18 to 30 years, were randomly assigned to one of three groups: low-intensity treadmill walking control (WALK) (n=10), low-intensity treadmill walking with blood flow restriction (BFR) (n-11), or a non-exercise control group (CON) (n=10). Subjects in the BFR and WALK groups walked on a treadmill at a speed associated with 45% VO_{2peak} for up to 20 minutes four days per week for 12 weeks. The BFR group wore 5 cm wide electronically monitored elastic pressure cuffs around their upper thighs during walk training. BFR cuffs were inflated to an initial pressure of 140 mmHg for the first four weeks, and then increased by 20 mmHg at week five (160 mmHg) and again at the start of week nine (180 mmHg). The CON group was asked not to change their normal physical activity levels or dietary habits over the duration of the training period. Baseline and post-testing measurements included blood sampling for the assessment of bone-specific alkaline phosphatase (Bone ALP) and tartrate-resistant acid phosphatase isoform 5b (TRAP5b); one repetition maximum (1RM) and maximal voluntary

contraction (MVC) strength testing for knee extension and flexion; graded treadmill exercise test (GXT) for the determination of VO_{2peak}; dual energy x-ray absorptiometry (DXA) to measure areal bone mineral density (aBMD) and body compsition; and peripheral Quantitative Computed Tomography (pQCT) to measure volumetric bone mineral density (vBMD) and bone area of the tibia as well as MCSA of the thigh and calf. **RESULTS**: A significant group x time interaction occurred for Bone ALP ($p=0.02$), as serum concentrations of Bone ALP were reduced in both BFR (-5.8%) and CON (-9.7%) groups post-training. Serum levels of TRAP5b and the ratio of Bone ALP to TRAP5b did not significantly change post-training. A significant group x time interaction was found for body weight ($p=0.034$). However, follow up analyses failed to find post-training group differences or within group changes over time ($p>0.05$). After analyzing percent change in body weight from baseline, significant group differences were observed between BFR (-0.8%) and WALK (2.4%) groups ($p=0.046$). A significant time effect ($p=0.02$) and group x time interaction ($p=0.002$) was observed for MCSA at the tibia 66% site. Follow up analyses revealed that MCSA significantly increased from baseline in both BFR (1.8%) and WALK (3.6%) groups ($p<0.05$). Significant time effects were found for MVC knee extension strength at joint angles of 30 degrees ($p=0.02$) and 60 degrees ($p=0.004$), with no differences between groups. A significant group x time interaction occurred for 1RM knee extension strength ($p=0.014$), with follow up analysis revealing a significant ($p=0.026$) increase in strength in the BFR group (4.5%) post-training. Significant main effects for time were found for trabecular bone content ($p=0.036$) and trabecular vBMD ($p=0.024$) at the tibia 4% site, both of which decreased over the study duration. Significant time effects were also

found for total bone content (p=0.036) and SSI (p=0.011) at the tibia 38% site as well as total bone content (p=0.043), total vBMD (p=0.029), total bone area (p=0.001), periosteal circumference (p=0.002), and endosteal circumference at the 66% site. Total vBMD at the 66% site decreased post-training, whereas the other variables with significant time effects increased over the study duration. **CONCLUSION**: Twelve weeks of walking with BFR resulted in reduced levels of bone formation with no change in bone resorption in young women. Additionally, BFR walking resulted in favorable neuromuscular changes.

CHAPTER I

INTRODUCTION

Osteoporosis, a disease of the skeleton characterized by decreased bone mass and strength with an increased risk for fragility fracture, is a serious public health problem with debilitating effects on health and daily function (56). In the United States, over 50% of women aged 85 years or older have osteoporosis (37). Osteoporosis is attributed to over 1.5 million fractures per year which result in 800,000 emergency room visits, 500,000 hospitalizations, 2.6 million physician visits, 180,000 nursing home placements, and up to $18 billion in healthcare costs each year (37). Following a hip fracture, the one-year mortality rate for individuals over the age of 50 has been estimated to be as high as 10-15 percent (67, 87). It has also been estimated that by the year 2020, over 14 million individuals will be osteoporotic and 47 million will be at risk for osteoporosis (38). As the population is living longer, there is increased demand for discovery of ways to prevent osteoporosis and the fractures associated with the disease. The discovery of new interventions to increase bone mass, especially in young adult women, may reduce the health care costs and increase quality of life in the postmenopausal years when osteoporosis is most prevalent.

Osteoporosis is diagnosed based on an individual's areal bone mineral density (aBMD). Areal BMD is the amount (grams) of bone mineral content (hydroxyapatite) per area of bone (cm^2) and has been shown to account for nearly 55-80% of whole bone strength (5). Areal BMD of the whole body, femur, lumbar spine, and radius can be determined by dual energy x-ray absorptiometry (DXA), which is a type of low-dose x-ray machine. The World Health Organization has developed a classification system for

the diagnosis of osteoporosis based on an individual's T-score, or the number of standard deviations the aBMD falls in relation to the average aBMD of a healthy Caucasian young adult female (52). A T-score of -2.5 or less, or 2.5 standard deviations below the young adult female average, is the clinical criterion for diagnosis of osteoporosis. Areal BMD has been shown to decline after menopause, and this bone loss has been closely associated with a decrease in circulating estrogen levels and an overall increase in bone turnover (26).

Bone turnover, or bone remodeling, is the metabolic process responsible for continuous renewal of bone to maintain mechanical integrity of the skeleton as well as aid in mineral homeostasis (44). Bone remodeling is achieved through a tightly coupled process of bone resorption and bone formation by the bone basic multicellular unit (BMU). Osteoclasts are responsible for the bone resorption phase and act to break down mineralized bone tissue. This is followed by differentiation and activation of osteoblasts, which serve to lay down new bone tissue followed by complete mineralization. Bone remodeling is hormonally and mechanically regulated. Parathyroid hormone (PTH), calcitonin, and 1,25-dihydroxyvitamin D3 (Vitamin D) directly and indirectly act to modulate bone remodeling to maintain serum calcium levels at 8.5-10 mg/dl (114). Parathyroid hormone is secreted in response to low levels of serum calcium and acts to increase bone resorption. In contrast, calcitonin is secreted in response to elevated levels of serum calcium and acts to decrease osteoclastic bone resorption, thus maintaining calcium homeostasis.

Mechanical loading is essential for proper bone growth and maintenance of skeletal integrity. Mechanically mediated bone turnover promotes site-specific

activation of the BMU to undergo adaptive changes and to repair damage. Increasing *in vivo* and *in vitro* evidence suggests that osteocytes are responsible for sensing bone strain caused by mechanical loading and subsequently relay these strains to activate other bone cells biochemically (49). According to Frost (32), bone modeling and remodeling are controlled by several mechanical thresholds where bone is either taken away, maintained, or added in order to meet the mechanical demands placed upon it. In this light, Frost alluded to the importance of the muscle and bone relationship, where he suggested that the largest strains on bone are derived from muscle contractions. In this regard, exercise that increases muscle strength and size should also influence bone metabolism and bone health.

Bone metabolism is a dynamic process, that when altered, can lead to bone loss or bone gain. The development of assays that measure biochemical bone turnover markers (BTM's) in serum or urine provide a dynamic view of the remodeling process. Their use in clinical settings has become valuable for monitoring treatment of osteoporosis as well as predicting long-term BMD changes (8, 42). Recently, BTM's have been used in predicting the long-term osteogenic potential of different types of exercise.

It has been appreciated for some time that mechanical loading through exercise positively affects bone mass. While peak bone mass has several determinants (lifestyle, genetics, etc.), it is generally accepted that mechanical loading during adolescence and early adulthood is critical for developing and maximizing peak bone mass, which subsequently helps to prevent bone loss later in life (78). Data from longitudinal studies investigating the efficacy of exercise to increase bone mass in young premenopausal

women have led the American College of Sports Medicine to recommend two general modes of exercise for bone health: high-impact exercises resulting in increased ground reaction forces (GRF's), and high-intensity resistance training (57). Generally, longitudinal studies employing progressive, high-intensity resistance training as well as high-impact aerobic-type exercises such as hopping and jumping have reported modest 1-3% increases in aBMD of the spine and/or hip in premenopausal women (57). The positive benefits of these types of exercise have also been alluded to in studies examining the effects of exercise on bone turnover markers. In a recent study by Lester et al. (68), increased levels of the bone formation marker, bone-specific alkaline phosphatase (Bone ALP), were reported in college-aged women after either eight weeks of high-intensity resistance training or resistance training combined with an aerobic program that included moderate to high-intensity jogging and interval running. In contrast, no changes in Bone ALP were seen in the aerobic-only group.

Recently, a novel form of training which involves the use of low-intensity exercise while restricting blood flow to the working muscle has been shown to increase muscle size and strength similar to that of traditional high-intensity resistance training (77). Blood flow restriction (BFR) training, also known as KAATSU training, involves the use of an electronically monitored and controlled pneumatic air pressure cuff which is placed around the most proximal portion of either the arms or legs and inflated during exercise. Most often, BFR cuffs have been used in conjunction with low-intensity (20-50% 1RM) resistance training and more recently, treadmill walking. Abe and colleagues (1) reported a significant increase in quadriceps and hamstring muscle cross-sectional area (MCSA) as well as dynamic and isometric knee extensor strength after

only three weeks of twice daily treadmill walking with BFR in college-aged men. Similar results were also found in older adults after 10 weeks of walking with BFR for 20 minutes per day (2). While the beneficial neuromuscular effects of this type of training are promising, little is known about the potential effects it may have on bone.

To date, one study has examined the effect of walking with BFR on BTM's in college-aged males. After three weeks of twice daily walking with blood flow restriction, resting levels of the bone formation marker Bone ALP were significantly elevated (10). This change was not observed in the control group who performed the same walking protocol but without BFR. Interestingly, this form of training does not meet the current criteria recommended by the American College of Sports Medicine for maintaining bone health (57). A low-intensity, functional type of exercise such as walking combined with BFR may provide an attractive alternative for individuals not able to perform the type of exercise currently recommended for bone health. However, the effects of walking with BFR on bone metabolism are not well described, and therefore, more evidence is needed to recommend its use.

Purpose

The purpose of this study was to examine the effects of 12 weeks of treadmill walking with and without blood flow restriction on biochemical markers of bone turnover in college-aged women. A secondary purpose was to examine changes in thigh and calf MCSA, muscle strength, aerobic capacity, and bone characteristics of the tibia following the intervention.

Research Question

1. Will 12 weeks of treadmill walking with and without blood flow restriction significantly alter the resting serum levels of the bone turnover markers bone-specific alkaline phosphatase (Bone ALP) and tartrate- resistant acid phosphatase isoform 5B (TRAP5b)?

Hypothesis

1. Twelve weeks of treadmill walking with blood flow restriction but not without will result in significantly elevated levels of Bone ALP. Resting levels of TRAP5b will not change following 12 weeks of treadmill walking with or without blood flow restriction.

Subquestions

1. Will 12 weeks of walking with and without blood flow restriction result in increased thigh and calf MCSA and leg bone free lean body mass?
2. Will 12 weeks of walking with and without blood flow restriction result in increased lower body strength?
3. Will 12 weeks of walking with and without blood flow restriction result in increased aerobic capacity?
4. Will 12 weeks of walking with and without blood flow restriction result in significant changes in bone characteristics of the tibia?

Subhypotheses

1. Twelve weeks of walking with blood flow restriction will result in increased calf and thigh MCSA and leg bone free lean body mass, with no changes occurring from walking without blood flow restriction.

2. Twelve weeks of walking with blood flow restriction will result in increased lower body strength, with no changes occurring from walking without blood flow restriction.
3. Twelve weeks of walking with blood flow restriction will result in increased aerobic capacity but will remain unchanged following 12 weeks of walking without blood flow restriction.
4. Twelve weeks of walking with blood flow restriction will result in positive bone adaptations at the distal tibia site with no change after 12 weeks of walking without blood flow restriction.

Significance

Walking with BFR has already been shown to provide beneficial neuromuscular adaptations in young men (1) and the elderly (2), and there is some evidence from a short-term (3 weeks) study of walking with BFR in young men that suggests favorable alterations in a bone formation marker (10). From what is currently known, high-impact activities such as jumping and high-intensity resistance training are thought to provide the most benefit to bone (57). However, these forms of training may not be appropriate for some populations. Walking is a very functional form of exercise that can be done by a wide range of individuals. If walking with BFR were to show a favorable change in the bone metabolic profile, it would provide an attractive alternative for individuals at risk for low bone mass, older adults, individuals recovering from injury/illness, and astronauts. However, in the current state, too few data exist to recommend its use.

Assumptions

1. All subjects gave maximal effort during baseline and post-testing.
2. Subjects did not exercise 48 hours prior to and were fasted overnight before baseline and post-training blood draws.
3. All subjects were taking oral contraceptives at the start and throughout the duration of the study.
4. Subjects in the non-exercise control group maintained their current level of physical activity throughout the study period and both exercise groups only participated in the exercise prescribed to them by the researchers.
5. Subjects answered questionnaires truthfully.

Delimitations

1. The findings of this study are only applicable to young women taking combination oral contraceptives. It is possible that the effects of exercise on BTM's could be different for non-oral contraceptive users, as combination oral contraceptives have been shown to alter bone metabolism.
2. Subjects are free of any physical or medical problems limiting them to exercise such as osteoarthritis, musculoskeletal injury, and/or cardiovascular disease.
3. Subjects are not currently active, defined as not doing structured exercise more than two days per week for the last three months.

Limitations

1. Exercise outside of the training program was not strictly monitored. However, all groups were asked not to participate in any exercise outside of the program and were reminded periodically throughout the intervention.

2. It is possible that sunlight exposure could have affected bone marker concentrations.
3. Combination oral contraceptive use throughout the study may have stopped or contraceptive method may have changed. However, in order to monitor oral contraceptive use, a simple questionnaire was given to each participant during each exercise session.
4. It is possible that weight loss may have occurred over the 12-week training period, which could affect some of the results including serum markers of bone turnover. A self-reported 3-day food log was used to monitor nutrient intake at baseline (week 1) and post-training (week 12). Vitamin supplementation was not monitored by the 3-day food log. Additionally, participants were asked not to purposely reduce their caloric intake or change their normal dietary patterns over the intervention period.
5. It is possible that bone marker concentrations were affected by normal diurnal rhythms. However, blood samples were obtained between 8:00 and 10:00 AM on both baseline and post-training collection days to minimize this effect.
6. Owing to the total length of the study and the time of year that it commenced, seasonal variation could affect serum bone marker concentrations.

Operational Definitions

Areal bone mineral density (aBMD) - The total grams of bone mineral per unit of bone area (g/cm^2), commonly assessed by dual energy x-ray absorptiometry.

Blood Flow Restriction training (BFR) - The use of an electronically monitored and controlled pneumatic air pressure cuff which is placed around the most proximal portion of an appendicular limb and inflated during exercise, also known as KAATSU training.

Bone-specific Alkaline Phosphatase (Bone ALP) - A bone-specific isoenzyme of alkaline phosphatase produced by the osteoblast. The function of bone alkaline phosphatase is not clearly understood, but it is thought to be involved in bone mineralization process (88).

Bone mineral content (BMC)--The total grams (g) of bone mineral within a scanned region

Bone Modeling – A process by which bone grows and becomes stronger through organized bone cell activity of osteoblasts and osteoclasts. It increases bone strength by increasing or adding mass and improving the existing geometry (88).

Bone Remodeling – The metabolic process mediated by osteoclasts and osteoblasts to renew bone and repair microdamage (88).

Dual Energy X-ray Absorptiometry (DXA) - Bone measurement modality that uses two contrasting x-ray beams to yield a two-dimensional representation of the skeleton. DXA calculates the attenuation values of photons that pass from the x-ray tube through the measurement site of interest. Outcome variables include bone mineral content (BMC) and areal bone mineral density (aBMD).

High-impact loading - Characterized by both high-rate and high-magnitude loading such as that experienced by bone during jumping and hopping, etc.

Mechanical Loading - Refers to the applied forces placed on the skeleton or individual bones via forms of physical movement or activity (33).

Oral Contraceptives – Also known as the birth control pill. Oral contraceptives are either a combination of estrogen and progestins or progestins alone in pill form used to inhibit ovulation by reducing the natural fluctuation of estrogen and progesterone hormones during the menstrual cycle.

Osteoblasts - Bone cells responsible for bone formation. Produce a bone matrix composed of collagen and other substances that ultimately becomes calcified (88).

Osteoclasts - A multinucleated bone cell responsible for the resorption of bone (88).

Osteoporosis - A disease of the skeleton characterized by low bone mineral density and micro-architectural deterioration, leading to bone fragility and increased risk of fracture (33).

Peripheral Quantitative Computed Tomography (pQCT) - Bone measurement modality that provides a three-dimensional representation of a particular site of interest. Measures the attenuation of radiation as it passes from the source to the site of interest. Outcome measures include BMC, vBMD, bone and muscle cross-sectional area, and bone thickness. Unlike DXA, pQCT has the ability to differentiate between types of bone and may be more sensitive to changes in bone due to physical activity (75).

Premenopausal - Women that have regular menstrual cycles that have not changed recently in duration (41).

Repetition maximum (RM) – A neuromuscular strength test usually assigned a specific number of repetitions i.e. 1RM, 5RM. The maximal weight at which an individual can produce a successful lift or lifts through the full joint range of motion.

Tartrate-Resistant Acid Phosphatase isoform 5b (TRAP5b) - Is typically expressed in mature osteoclasts and is proportionate to osteoclast activity as well as representative of osteoclast number (88).

CHAPTER II

LITERATURE REVIEW

Introduction

Osteoporosis is a bone disease characterized by low bone mass and micro-architectural deterioration resulting in bone fragility and an increase in the susceptibility for trauma-induced fracture (45). Essentially, osteoporosis manifests as a silent disease until an osteoporotic fracture occurs. According to the Surgeon General (38), four out of ten women over the age of 50 will experience an osteoporotic fracture of the hip, spine, or wrist and the overall incidence of osteoporotic fractures are on the rise. Osteoporotic fractures are a serious and life-threatening problem. Approximately one in five patients over the age of 50 who experience hip fracture will die within one year (74) and one third of those who experience hip fracture will require nursing home assistance (60). Besides the devastating clinical consequences to the patient and their family, osteoporotic fractures also result in a substantial financial burden. It has been estimated that the direct costs associated with osteoporotic fractures is approximately 17 billion per year with projections close to 40 billion by the year 2040 (60).

At menopause, cessation of sex hormone production is associated with an increase in bone turnover and accelerated bone loss in women. Because low peak bone mass is a risk factor for osteoporosis, it is generally accepted that maximizing peak bone mass through adequate nutrition and physical activity in the adolescent and early premenopausal years may help to prevent bone loss associated with the menopause (60). In this regard, exercise interventions that result in favorable bone adaptations in premenopausal women could potentially help to decrease the occurrence of osteoporosis

and osteoporosis-related fractures later in life. Currently, exercise resulting in high ground reaction forces as well as high-intensity resistance training is recommended for bone health (57). However, these types of exercise may not be appropriate for all individuals, especially those with already low bone mass or recovering from a de-conditioned state.

This literature review will examine bone structure and physiology with emphasis on the remodeling process and its regulation, mechanical loading and bone mechanotransduction mechanisms, biochemical markers of bone turnover, the effects of exercise on biochemical markers of bone turnover and aBMD in premenopausal women, as well as a novel form of exercise training which may have important implications for bone health.

Bone Structure and Physiology

The skeleton is a remarkable organ that fulfills multiple functions including support and protection of vital organs, acting as a lever for locomotion, and is the largest storage for calcium and phosphate needed for the maintenance of mineral homeostasis. Approximately 70% of bone is composed of inorganic and 30% organic material (88). The vast majority of the organic matrix consists primarily of Type I collagen fibers and non-collagenous proteins. Also included in the bone matrix are several types of bone cells, cytokines, and growth factors which are necessary for maintaining bone homeostasis. The inorganic portion of bone is composed primarily of hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$] which precipitates on the organic matrix providing bone with its structural rigidity and strength (114). Together, the organic and inorganic components combine to form two main types of bone.

The majority of the human skeleton is composed of the dense cortical bone while the more porous trabecular bone only accounts for about a fifth of the total skeleton (16). However, different bones will have different ratios of cortical and trabecular bone dependent on the bone's primary function. While cortical and trabecular bone contain the same general components, their structure, mechanical and metabolism are inherently different. Cortical bone is dense and compact and is resistant to bending and torsional forces (44). These characteristics are in part due to its structural design. The haversian system of cortical bone is composed of several layers of densely packed concentric lamellae which form a cylindrical canal that houses vessels, nerve, connective tissue, and bone cells (114). Trabecular bone is less dense but more elastic than cortical bone. It consists of vertical and horizontal lamellae that form a latticework of struts shaped as plates and rods. This design allows trabecular bone to resist compressional forces by distributing the stress throughout the latticework (22). Trabecular bone has a large surface area accessible to osteoclastic resorption and is metabolically more active in the maintenance of bone mineral homeostasis than cortical bone (88).

Bone is a dynamic and active tissue that undergoes continuous turnover, repair, and structural micro-cellular change throughout the lifespan. It is estimated that about 10 percent of the human skeleton is renewed every year through bone remodeling (114). Bone remodeling is achieved by a temporary bone structure called the basic multicellular unit (BMU). Each BMU consists primarily of the bone resorption cells osteoclasts and bone formation cells osteoblasts. Osteocytes, which are differentiated mature osteoblasts, are also involved in the remodeling process. Osteoblasts are derived

from undifferentiated mesenchymal stem cells (MSC). Their differentiation from MSC's to pre-osteoblasts and ultimately mature osteoblasts require the coordinated interaction of multiple endocrine, paracrine, and autocrine factors (18). Pre-osteoblasts as well as mature osteoblasts have several important roles in the remodeling process including expression and regulation of osteoclastogenic factors, production of bone matrix proteins, and bone mineralization (90). Osteoclasts are large multinucleated bone cells responsible for the bone resorption phase in bone remodeling. Osteoclast precursor cells differentiate from hematopoietic stem cells and are of the monocyte-macrophage lineage (16). Two local cytokines are responsible for osteoclast differentiation including Macrophage Colony Stimulating Factor (M-CSF) and receptor activator of NF-$_k$B ligand (RANKL). M-CSF is essential for the proliferation, differentiation, and survival of osteoclast precursor cells (16). RANKL belongs to the tumor necrosis factor (TNF) receptor super family and is critical for terminal differentiation of osteoclast precursors to mature osteoclasts (66). Osteoclasts secrete hydrogen ions as well as collagenolytic enzymes to aid in the breakdown and digestion of the bone matrix during bone resorption (16). Osteocytes are terminally differentiated osteoblasts that become engulfed in the unmineralized osteoid matrix during bone deposition. They account for 90-95% of all bone cells. Osteocytes are housed in fluid-filled cavities within the mineralized matrix called lacunae and have dendritic processes that extend through tiny canals called canaliculi which allow direct interaction with other osteocytes, osteoblasts on the bone surface, and bone precursor cells in the marrow (12). Due to their ubiquity, location, and extensive dendritic network, osteocytes are thought to be the bone cell

responsible for sensing mechanical strain and converting it to a useful biochemical signal for the (re)modeling process (12).

Bone remodeling/turnover is the process by which bone is renewed to maintain skeletal strength and regulate mineral homeostasis. Four distinct phases encompass bone remodeling: activation, resorption, reversal, and formation. Together, these phases occur over a 3-6 month span (16). The activation phase involves exposing the bone surface for osteoclast attachment as well as recruitment of preosteoclasts to the resorption site. Once at the resorption site, preosteoclasts anchor to adhesion sites within the exposed bone matrix and start the resorption process. The resorption phase involves proliferation, differentiation, and activation of mature osteoclasts by osteoblastic regulatory factors (44). The osteoclast forms a sealing zone along the bone surface and secretes hydrogen ions which mobilize bone mineral. The remaining organic matrix is degraded by the osteoclastic secreted enzymes cathepsin K and tartrate-resistant acid phosphatase (90). Following osteoclast-mediated resorption, the reversal phase acts to prepare the newly resorbed bone surface for bone deposition by osteoblasts. This preparation is accomplished, in part, by a "reversal" cell whose lineage has yet to be identified (90). The final phase of bone remodeling is formation of newly synthesized bone. Preosteoblast cells are recruited to the resorption pit to undergo differentiation into mature osteoid-secreting osteoblasts (44). Mature osteoblasts synthesize and secrete type I collagen along with other bone matrix proteins which fill the resorption cavity. This is followed by mineralization within and around the newly formed osteoid matrix.

The regulation of bone remodeling is mediated either directly or indirectly by the actions of several hormones. A serum calcium level of 8.5-10 mg/dl is tightly regulated by the secretion of parathyroid hormone (PTH), calcitonin, and vitamin D (114). When serum calcium levels are suppressed, calcium sensitive receptors on the parathyroid cell membrane respond by signaling an increase in secretion of PTH (13). In an attempt to increase serum calcium, PTH exerts its effects on the bones, kidneys, and intestine. In bone, PTH receptors are expressed by osteoblasts and osteocytes and activation of the receptor by PTH increases the expression of the osteoclastic regulator, RANKL (114). Increased expression of RANKL results in activation of osteoclasts to increase bone resorption resulting in extracellular calcium release. When serum calcium levels become too high, calcitonin is secreted from the C cells of the thyroid gland which targets bone and kidneys. In bone, calcitonin acts directly on osteoclasts by causing loss of the ruffled border and its capacity to secrete degradation enzymes(14). Ultimately, calcitonin acts to decrease bone resorption.

It is well known that estrogen and its receptors play an important role in the regulation of bone remodeling and that the loss of estrogen during the menopause results in increased bone resorption and subsequent bone loss (125). However, the exact mechanism(s) by which estrogen exerts its positive bone effects are still under intense investigation. Generally, estrogen has an effect on cell viability. There is evidence that suggests estrogen promotes osteoblast and osteocyte survival as well as apoptosis and anti-proliferation of osteoclasts (125). Nakamura et al. (80) have recently shown that female mice with selective knockout of the osteoclast estrogen receptor alpha (ERalpha) have greater trabecular bone loss compared to their wild type

counterpart due to increased osteoclastogenesis and thus bone resorption. In addition, the administration of estrogen in the ERalpha knockout mice did not result in bone recovery. Besides the effects of estrogen on cell viability and apoptosis, It has also been suggested that estrogen acts to determine the "set point" for the bone response to mechanical loading (33). Work by Devlin and Liberman (21) has shown that young sheep administered different levels of estrogen have a dose-dependent response of bone growth when exposed to the same exercise regimen. Specifically, sheep administered a high dose of estrogen had greater bone accrual after 45 days of exercise than did sheep with normal estrogen or decreased estrogen levels. In support of these data, Lanyon's group (65) has shown that wild-type mice have a three-fold greater bone growth response to ulnar mechanical loading than their ERalpha knockout counterparts. In cell culture studies, Lanyon (65) has also shown that osteoblast-like cells deficient in ERalpha have reduced proliferative capacity after being subjected to mechanical stretching. More recently, Lanyon's group (61) has shown that ERalpha aids in the shuttling of beta-catenin into the nucleus during mechanotransduction, thereby increasing activation of the Wnt/beta-catenin bone formation pathway.

Mechanical Loading

Mechanical loading of bones is vital for normal skeletal maturation and for maintaining bone strength throughout the lifetime (34). Mechanically mediated bone turnover promotes site-specific activation of the BMU to undergo adaptive changes and to repair damage. The importance of skeletal loading has been appreciated for some time, as Julius Wolff in the late 1800's realized that bone growth and remodeling seemed to be in response to the physical demands placed upon it (120). This

phenomena was expanded upon by Harold Frost (32) who proposed the "mechanostat" theory of bone modeling and remodeling in response to the mechanical environment. The mechanostat theory suggests that bone mass and structure are regulated by several mechanical thresholds in which mechanical signals above or below a certain threshold result in either bone being taken away or added. Frost also suggested that the largest mechanical loads acting upon bone are from muscle contractions; hence whole bone strength adapts to meet the demands of increased muscle strength and size. Frost's theory has been supported by several studies that have found surrogates of muscle strength such as muscle cross-sectional area and appendicular bone free lean body mass to consistently be the best predictors of indices of bone strength (27, 73, 101). The importance of mechanical loading to the skeleton has been supported by several human studies. Loss of bone mass has been reported in individuals confined to bed rest (59), in limbs that are immobilized due to injury (117), and in astronauts exposed to weightless environments (64). Alternatively, increased bone mass has been reported in the dominant limbs of ten-pin bowlers (124), tennis players (43), and gymnasts (121). Most experimental evidence that exercise or mechanical loading can increase bone mass comes from controlled trials using animal models. In this regard, Turner's (112) work led him to propose a set of criteria necessary for bone to respond to loading. First, the loading must be dynamic, as static loads are not recognized by the mechanosensing machinery. Second, extending loading duration does not result in increased bone mass. Therefore, short duration loading periods are beneficial for bone. Lastly, the mechanosensing machinery accommodates to routine loading and therefore, the mechanical loading should deliver "abnormal" strains. While experimental animal

studies have confirmed these criteria, human exercise interventions to increase bone mass have not been as successful. One reason for this observation may be a lack of understanding in the exact mechanism of how bone's mechanosensing machinery interprets mechanical strain and changes it to an anabolic biochemical signal.

The location, abundance, and cell-interconnectivity of osteocytes make these cells an ideal candidate for transducing mechanical strain into a biochemical signal (84). Tatsumi and colleagues (111) recently showed that selective osteocyte ablation in mice resulted in osteoporosis due to faulty mechanotransduction. Although the osteocyte is becoming more accepted as the mechanosensing cell in bone, the exact mechanism by which it responds to strain has yet to be elucidated and is an ongoing area of research. Most of what is known has been derived from isolated cell culture studies as well as theoretical mathematical modeling. One hypothesis is that the osteocyte detects bone deformation via direct cellular strain from the matrix (12). The second hypothesis, which is becoming more accepted, is that mechanical strain on the bone generates a pressure gradient in which lacunar-canicular interstitial fluid is propelled from the area of deformation to an area of tension (31). Theoretically, the increased fluid flow from dynamic loading would cause fluid shear stress across either the cell body or dendritic processes or both (12). While the exact mechanism is still unknown, Wang and colleagues (116), through examination of the lacunar-canicular system with electron microscopy, have proposed that the fluid shear stress activates integrins that connect the dendritic process membrane to the canicular wall. Wang's elastohydrodynamic model for integrin-based signaling would allow for amplification of cellular-based strain. This is a very important feature of the model, especially since in vitro cell-strain studies

indicate that a strain magnitude of at least 0.5% (5000 microstrain) is required at the cellular level to initiate a biochemical response (123), yet whole bone strain caused by movement rarely exceed 0.2% (2000 microstrain) (29, 30). Even though Wang's theoretical model provides indirect evidence for the fluid flow hypothesis, direct in vivo evidence of increased fluid flow from external mechanical loading has been lacking. However, the fluid flow hypothesis was strengthened when Price and colleagues (89), for the first time, were able to show an increase in lacunar-canicular fluid flow from in vivo mechanical loading. Through fluorescence recovery after bleaching and synchronized mechanical loading via cyclic compression, Price and colleagues were able to definitively show that moderate mechanical loading increased fluid flow by 31% in rat tibia. Importantly, Price and colleagues also estimated that the magnitude of fluid shear stress produced was similar to that which triggers biochemical reactions in vitro.

Since the isolation and establishment of osteocyte-like cells (MLO-Y4) (54), in vitro studies of these cells exposed to fluid shear stress have obtained some important data regarding biochemical pathways in the control of bone remodeling. Osteocyte-like cells exposed to pulsating fluid shear stress have been shown to increase expression of prostaglandins (PGE2) and nitric oxide (NO) as well as increased phosphorylation of GSK-3beta and other beta-catenin target genes involved in the Wnt/beta-catenin bone formation pathways (12). In vivo studies have started to confirm these findings. Kramer and colleagues (58) found that osteoclast number and activity were increased in osteocyte-specific beta-catenin deficient mice. In addition, the RANKL/OPG ratio was increased and the animal's cancellous bone mass was almost non-existent. Robling and colleagues (95) found that osteocyte sclerostin expression was reduced in rodent ulna

exposed to cyclic mechanical loading but increased expression was found in rodents exposed to hind limb suspension. Sclerostin is only expressed by osteocytes and is a powerful inhibitor of the Wnt/beta-catenin bone formation pathway (95). Taken together, these in vitro and in vivo studies provide some compelling evidence into the mechanisms by which osteocytes control mechanical loading-mediated bone remodeling.

Biochemical Markers of Bone Turnover

Bone mineral density measured by DXA is considered the gold standard for bone health and skeletal status. While BMD is clinically useful, it is a static measurement and tells us little about the dynamic process of bone turnover. In the last 20 years, assays capable of identifying blood serum or urine-derived biomarkers of bone formation and resorption have been developed. These markers provide a dynamic view of the bone remodeling process and are useful in assessing the remodeling response to exercise and pharmacological interventions. In general, bone turnover markers (BTM's) are classified as either markers of bone formation or bone resorption. Bone formation markers are either direct or indirect products of osteoblast development, function, or mineralization (20). They include bone-specific alkaline phosphatase (Bone ALP), osteocalcin (OC), and N-terminal and C-terminal procollagen type I propeptides (PINP, PICP). Bone resorption markers are either degradation products of bone matrix or enzymes secreted from osteoclasts (107)**.** They include N and C-Terminal cross-linking telopeptides of Type I collagen (NTX-1, CTX-1), C-terminal cross-linking telopeptides of type I collagen generated by metalloproteinase (ICTP),

deoxypyridinoline and pyridinoline (DPD, PYD) and tartrate-resistant acid phosphatase isoform 5b (TRAP5b).

In order for the use of BTM's to be clinically useful, their change to an intervention should be predictive of long-term changes in BMD. Greenspan and colleagues (42) conducted a three-year study on women taking hormone replacement therapy, alendronate, or a combination of both. They found that women who experienced the greatest decreases in NTX-1 measured at 6 months also experienced the greatest increases in aBMD at 3 years. Bauer et al. (8) conducted a study to investigate the effects of PTH treatment over one year in osteoporotic women. Blood samples were obtained at baseline and at 1 and 3 months after treatment. BMD of the spine and hip were determined using DXA and QCT at baseline and after one year of treatment. Treatment with PTH resulted in increased levels of the bone formation marker PINP as well as increases in volumetric BMD of the spine. Additionally, each standard deviation increase in the 3-month change of PINP was associated with a 21% greater increase in spine trabecular vBMD. While these studies found significant relationships between BTM's and long-term changes in BMD, it must be noted that the BTM response is highly variable and proper care to standardize measurements must be taken.

BTM's can be influenced by several biological factors including time of day (diurnal variation), seasonal changes (time of year), feeding status, menopausal status, and menstrual cycle phase (107). Garnero and colleagues (36) conducted a study investigating menopausal status on multiple BTM's of formation and resorption. 653 women free of medications known to affect bone metabolism were separated into four groups based upon their menopausal status: premenopausal, perimenopausal 1,

perimenopausal 2, and postmenopausal. The menopause transition resulted in a 37-53% increase in bone formation markers and a 79-97% increase in resorption markers. Additionally, these markers did not decrease with age once menopause was reached. The researchers concluded that a high turnover rate associated with the menopause may be a determinant of bone loss. The menstrual cycle has also been shown to affect the levels of certain bone markers. Zittermann and colleagues (126) measured bone formation and resorption markers in ten premenopausal women with normal menses during the 4 phases of the menstrual cycle. They found that both formation and resorption markers fluctuated in a cyclic pattern. The researchers concluded that the fluctuations in BTM's were likely due to the fluctuations in estrogen throughout the menstrual cycle. Oral contraceptives have also been shown to alter BTM's. Nappi and colleagues (81) conducted a 12-month randomized controlled trial on the effects of low-dose and ultra-low-dose oral contraceptives on BTM's and aBMD in premenopausal women . Sixty premenopausal women with normal menses were randomly assigned to either a low-dose oral contraceptive (20 micrograms ethinyl estradiol), ultra-low-dose oral contraceptive(15 micrograms ethinyl estradiol), or non-contraceptive control group. Blood and urine samples were taken at baseline, 3, 6, 9, and 12 months and analyzed for OC, DPD, and PYD. Areal BMD of the spine was analyzed at baseline and 12 months. Both oral contraceptive groups showed significant reductions in bone resorption markers at 6, 9, and 12 months compared to the control group. No change in OC or aBMD was found. Taken together, the results of these studies suggest that menstrual history and status, menstrual phase, and oral contraceptive use must be considered when measuring changes in BTM's in women.

Exercise Interventions and BTM's in Premenopausal Women

Interventions designed to test the efficacy of different types of exercise to stimulate bone formation or slow its loss typically last 6-36 months due to bone mass changes occurring from a slow metabolic process (57). In this regard, testing the osteogenic potential of different types of exercise by measuring chronic changes in BTM's is becoming more popular. To date, few studies have measured chronic changes in BTM's in premenopausal women after exercise training. Lohman and colleagues (72) studied the effects of an 18-month progressive resistance training program on lean muscle mass, aBMD, and serum OC in premenopausal women with normal menses not taking oral contraceptives. The resistance training program consisted of 12 upper and lower body exercises designed to target major muscle groups and were performed at an intensity of 70% 1RM which was periodically reassessed and progressively increased to 80% 1RM by the end of the study. Blood samples and measurements of hip and spine aBMD were taken at baseline, 5, 12, and 18 months. Blood samples were drawn in the morning after an overnight fast during days 1-5 of the menstrual cycle. After 5 months of training, serum OC increased in the exercise group and remained elevated at 12 and 18 months but did not change in the control group. Furthermore, the exercise group also experienced increases in femoral trochanteric and lumbar spine aBMD compared to control. Increased bone formation markers were also reported by Shibata and colleagues (103) after a one-year walking combined with jumping intervention in premenopausal women. 43 premenopausal women were assigned to either a walking group or a walking combined with jumping group. Both groups were assigned to walk 10,000 steps per day with the walk-jump group assigned 10 maximal jumps per day in addition to the

10,000 steps. Serum levels of bone formation markers Bone ALP and OC as well as the resorption marker NTX were measured at baseline and after one-year in both groups. Bone ALP was significantly elevated from baseline in both groups. However, Bone ALP was higher in the walk-jump group compared to the walk-only group. Serum levels of CTX were unaffected by exercise in either group. The researchers concluded that adding exercises with higher ground reaction forces to a walking program had a more positive influence on bone metabolism than walking alone. Adami and colleagues (4) conducted a two-part investigation to study the effects of physical activity on BTM's. First, 530 premenopausal women with normal menses not taking oral contraceptives were enrolled to take part in a cross-sectional investigation to determine the relationship between basal levels of BTM's, self-reported physical activity levels, and aBMD of the femoral neck and lumbar spine. All women had their blood drawn between 7:00 and 8:00 AM after an overnight fast which was analyzed for OC, PINP, and CTX. The results of this observational study found that the BTM's OC and PINP, but not CTX, were significantly associated with level of physical activity. Additionally, spine and hip aBMD were related to physical activity levels, but only spine aBMD showed a statistically significant change. With a subset of the women in the cross-sectional study, the same researchers investigated the effects of a one-month moderate intensity weight-bearing aerobics program on BTM's. 24 sedentary women in the exercise group and 18 age-matched controls participated in the intervention study. The exercise sessions were conducted 3-4 days per week lasting approximately 90 minutes and consisted of moderate intensity weight-bearing aerobic activities combined with spine flexion and extension strength exercises. After 4 weeks of exercise training, both

groups had their blood drawn and reanalyzed for levels of OC, PINP, and CTX. A significant 25% increase in both OC and PINP occurred in the exercise group with no change in non-exercise controls. The bone resorption marker CTX did not change in either group. From the evidence in both the intervention and cross-sectional study, the researchers concluded that increased physical activity is associated with a clear effect on bone formation markers and further explains the crucial role of physical activity toward bone health. Recently, Lester and colleagues (68) conducted an investigation to determine the influence of different modes of exercise on BTM's after training. 58 premenopausal sedentary women were assigned to one of four groups: aerobic, resistance, combination, or non-exercise control. All three exercise groups met three days per week for 8 weeks. The aerobic group performed 30-60 minutes of either jogging or running at moderate to high intensities (70-85% HR_{max}). The resistance training group performed 6-8 exercises targeting the main muscle groups of the upper and lower body. Load and repetition was altered in a non-linear periodized fashion at each resistance training session over the 8-week period. The combined group performed both the resistance and aerobic regimens in a single session. The resistance training portion was always performed first to maximize force production. All participants had their blood drawn after a 12-hour fast at baseline, 4 weeks, and 8 weeks post-training to determine serum levels of the bone formation markers Bone ALP and OC as well as the resorption markers TRAP5b and CTX. Dietary intake was recorded at baseline and was used to ensure that the same meal was consumed the day before the 4 and 8-week blood draws. In order to minimize biological variability, both 4 and 8-week blood draws were taken 48 hours after exercise and during the same approximate phase of the menstrual

cycle. After 8 weeks of training, serum levels of BAP and OC were significantly increased in the resistance and combined training groups but not in the aerobic or non-exercise control. No training-related changes in serum CTX or TRAP5b were observed. The authors concluded that exercise programs with a resistance aspect are more effective at elevating serum markers of bone formation than programs consisting only of aerobic training.

With the current data available, it is difficult to definitively determine the effects of exercise training on BTM's in premenopausal women. With regard to the studies discussed, the evidence presented suggests that exercise resulting in high impact ground reaction forces, moderate to high-intensity resistance training, or a combination of the two can favorably induce changes in the bone metabolic profile. Whether these exercise-induced changes lead to future gains in bone mass is not known. Because of the variability associated with BTM's, it is absolutely necessary to standardize measurement protocols. Menstrual status, menstrual phase, and oral contraceptive use all affect BTM's and must be considered when designing studies to investigate the effects of exercise on BTM's in premenopausal women.

Exercise Interventions and BMD in Premenopausal Women

The sensitivity of the skeleton to mechanical loading through exercise is age-dependent and it is widely accepted that the skeleton reaches its peak bone mass by the third decade (57). Research has shown that bone in young premenopausal women responds differently to exercise compared to their postmenopausal counter-parts and this period in life may be the final opportunity to enhance bone mass before the menopause (7). In humans, BMD is a widely used measure of bone strength and has

been estimated to account for approximately 60% of the variance in bone strength (57). Because low BMD is a risk factor for trauma-related fractures, determining an exercise prescription that enhances BMD and muscular strength in premenopausal women could potentially minimize bone loss and thus fracture risk in the later years. A multitude of evidence from cross-sectional studies suggest that young women who participate in regular high-intensity resistance training (19, 46-48) or exercise that results in high-impact forces such as gymnastics (17, 25, 93) have stronger bones than their sedentary age-matched counterparts. However, longitudinal randomized controlled trials investigating the effects of different modes of exercise on bone mass in sedentary premenopausal women have reported only modest increases in aBMD (1-3 %), if any.

Resistance training as a means to increase bone mass in premenopausal women has been studied by several researchers. Snow-Harter and colleagues (105) conducted an 8-month study of the effects of resistance training on spine and hip aBMD in college-aged women. The resistance training protocol consisted of 14 exercises designed to stress the major muscles of the upper and lower body and was completed three days per week. Training intensity at the beginning of the study was 65% 1RM and was progressively increased to 85% 1RM by the end of the study. In comparison to the non-exercise control group, the resistance training group significantly increased (1.2%) lumbar spine aBMD. However, hip aBMD was unchanged. Similar findings were reported by Lohman and colleagues (72) after an 18-month progressive resistance training program in premenopausal women. Their training program, performed three days per week, consisted of 12 upper and lower body exercises designed to target major muscle groups. The intensity commenced at 70% 1RM which was periodically

reassessed and progressively increased to 80% 1RM by the end of the study. Hip and spine aBMD were taken at baseline, 5, 12, and 18 months. The exercise group experienced a significant increase in lumbar spine (1.9%) and femoral trochanter (2.0%) aBMD compared to non-exercise controls. Others researchers investigating resistance training did not find significant changes in aBMD. The studies by Sinaki et al. (104) and Gleeson et al. (39) both failed to show significant improvements in hip or spine aBMD in the exercise group compared to the control after resistance training exercise. This may be due to the prescribed intensity of exercise employed in their studies. Sinaki et al. reported their exercise as "non-strenuous" and back extension exercises were performed for 10 repetitions at 30% of 1-RM. Similarly, Gleeson et al. prescribed a "goal" intensity of 60% 1-RM for the eight exercises performed in their study. Taken together, the results of these studies suggest that resistance training-induced bone mass gain may be intensity-dependent. Thus, intensities that elicit greater bone-loading magnitudes are needed to augment bone accrual.

Exercises that result in increased ground reaction forces (GRF's) have also been hypothesized as osteogenic. Activities such as high-impact aerobics and jumping can result in GRF's two to six times bodyweight (15, 23). Bassey and colleagues (7) conducted a 6 month study investigating the effects of jump training on femoral neck, femoral trochacter, and lumbar spine aBMD. 55 premenopausal were randomly assigned to a jumping group or non-jumping control group. The jumping program consisted of 50 maximal double-leg jumps (5 sets X 10 jumps) six days per week. GRF's were sampled throughout the study with a force plate and determined to be, on average, three times body weight. At the conclusion of the study, the jump group

significantly increased aBMD of the femoral trochanter (2.8%) and femoral neck, with no change in the lumbar spine. Similar results have been reported by other researchers employing high GRF exercise interventions (28, 115). Interestingly, these researchers also only found significant increases in hip aBMD but not lumbar spine, suggesting that high GRF activities may result in site-specific bone adaptations. To test this hypothesis, Winters-Stone and colleagues (118) conducted a 12 month study investigating the site-specific effects of jump training combined with either lower or upper body resistance exercise on spine and hip aBMD. Women were assigned to one of three groups: Lower training, Lower + Upper training, or non-exercise control. Both training groups were required to complete 9 sets of 10-12 jumps of varying height and direction as well as 9 sets of 10-12 repetitions of squats and lunges. The Lower + Upper training group performed 6 upper-body exercises for 3 sets each at an intensity of 8-12 RM. The resistance was progressively increased throughout the training duration. Both the Lower and Lower + Upper training groups significantly increased aBMD of the femoral trochanter (2.7% and 2.2%, respectively), but only the Lower + Upper group increased aBMD of the lumbar spine. The results of this study suggest that bone adaptation to exercise training is site-specific. Therefore, a combination of high-impact lower body exercises and upper-body resistance exercises would be optimal for augmenting bone accrual at multiple skeletal sites.

Recently, there has been some compelling evidence that high magnitude and/or high-impact loading may not be needed to improve or preserve bone mass. Using a low-magnitude, high-frequency mechanical stimulus, Rubin and colleagues (98) reported significant increases in trabecular bone quantity and quality in the hind legs of

sheep exposed to 30 Hz vibration (approximately 5 microstrain) 20 minutes per day five days per week over 12 months. These data in sheep suggest that low magnitude, high frequency loading delivers an osteogenic response to bone. Interestingly, muscle contractions have been shown to occur at frequencies up to 30 Hz while typical locomotor patterns such as walking deliver frequencies to the skeleton on the order of 1-3 Hz (113). To test the effects of high-frequency, low-magnitude loading on aBMD in humans, Beck and colleagues (9) recruited seven premenopausal women with known low aBMD to undergo 12 months of whole body vibration training at 30 Hz (approximately 5 microstrain). Subjects were instructed to stand on the vibration platform every day for 10 minutes in the morning and at night. aBMD of the hip, spine, and radius was assessed at baseline, six, and 12 months. Following the year-long intervention, aBMD of the non-dominant proximal femur increased 2%. No significant change was found at any other sites. It must be noted that there are many methodological flaws inherent in this study. The small number of subjects and a lack of a non-exercise control group undermine the beneficial results reported. Because of the many short comings of this study, it is not possible to draw any definitive conclusions on the effects of this novel type of mechanical loading. Nonetheless, if found to be beneficial in a randomized controlled trial, an intervention of this type could prove to be a safer option for frail individuals at risk for fracture.

Blood Flow Restriction Training

In the last ten years, blood flow restriction (BFR) training has gained considerable attention in the United States. This training method, also known as "KAATSU" training, has been popular in Japan for over 30 years (100). BFR training

involves the use of an electronically monitored and controlled pneumatic air pressure cuff which is placed around the most proximal portion of an appendicular limb and inflated during exercise. Most often, BFR cuffs have been used in conjunction with low-intensity (20-50% 1RM) resistance training. The cuff pressure used varies, but is often above systolic brachial pressure (140-280 mmHg). The cuffs are designed to occlude venous return as well as reduce arterial flow to the exercising limbs, thus causing venous pooling. Two studies investigating cuff pressure and arterial blood flow found that a pressure of 160-200 mmHg around the proximal thigh reduced arterial flow 30-60% (51, 108), but the degree of blood flow restriction is also dependent on the size of the underlying tissue (70, 102) .

The safety of BFR training has been a large concern with its use, as the idea of reducing blood flow to working muscle seems not only counter intuitive, but potentially harmful. On the contrary, the use of tourniquets in the field of surgery is a widely accepted practice and the safety of its use has been well documented. Studies show that tourniquets inflated at 350 mmHg are safe for use in duration of 90 minutes with no measurable muscle damage (99). Additionally, a national survey in regards to safety and adverse events was sent to 105 facilities in Japan that regularly use BFR training and resulted in a response of approximately 13,000 people, ranging from under the age of 20 years to 70 years (79). The number one adverse side effect reported was subcutaneous bruising (13.1%) at the sight of the cuff as well as numbness (1.3%). However, numbness usually subsided upon release of the cuff.

The novel finding that BFR combined with low-intensity (20-50% 1RM) resistance training increases muscle strength and muscle size comparable to traditional

high-intensity (75-85% 1RM) resistance training has been supported by a number of studies (3, 35, 85, 110). Surprisingly, one investigation showed marked muscle hypertrophy measured by MRI in as little as six days (35). These data are intriguing in that this training method might provide a safer alternative compared to high-intensity training for older individuals or those not able to lift heavy loads. Several hypotheses exist as to how such low intensities combined with BFR result in hypertrophy, including increased muscle activation (110, 122), increased secretion of anabolic hormones (76, 86, 91, 108, 109), and more recently, through fluid shifts and cell swelling(69). However, the exact mechanism has yet to be elucidated.

More recently, BFR has been used in combination with more functional exercise such as walking. Abe and colleagues (1) first investigated the use of BFR walking in college-aged males. BFR walk training was conducted two times per day, six days per week for three weeks in 18 men. Restriction cuffs were worn bilaterally at the proximal thigh and commenced at 160 mmHg on day 1. Pressure was increased 10 mmHg each day until a final pressure of 230 mmHg was reached (day 8) and maintained for the duration of the study. Both subjects in the BFR walk group (n=9) and non-BFR walk group (n=9) walked at 50m/min for five two-minute bouts with one-minute rests in between. Total time under restriction for the BFR group was 17 minutes for each session. After 3 weeks of training, subjects in the BFR group significantly increased MRI-derived hamstring (7.6%) and quadriceps (5.7%) MCSA while no change occurred in the walk control group. In addition, dynamic (8.3%) and isometric (10.5%) knee extension strength increased in the BFR group with no change in controls. Similar findings have since been reported in older adults (2, 82). Both of these studies utilized

BFR with low-intensity (45% heart rate reserve, 67 m/min) treadmill walking. Unlike the previous study in young males, these studies used a 20-minute continuous bout with no intermittent recovery periods. Again, both these studies reported significant increases in thigh MCSA and lower body strength compared to non-BFR walking control groups after 6 and 10 weeks of training, respectively.

To date, no studies have been conducted on the long-term effects of BFR training on bone adaptations and only three studies have reported the effects of BFR on changes in BTMs. Bemben et al. (11) conducted a repeated-measures crossover-design study on the acute effects of a single bout of low-intensity resistance training with and without BFR. Nine college-aged men performed an initial warm-up set of 30 repetitions at 20% 1RM. Following warm up, 3 sets of 15 repetitions separated by 30 seconds of rest were completed. This protocol was followed for both leg extension and leg curl exercises in a BFR condition and non BFR condition. Condition and starting exercise were randomly assigned with conditions being performed at least 48 hours apart. BFR cuff pressure was set at 180 mmHg and maintained for the duration of both exercises. Baseline blood samples were taken in the morning after an overnight fast followed by samples immediately and 30 minutes after exercise which were analyzed for Bone ALP and NTx. No change in serum levels of Bone ALP were reported at any time point or condition. However, NTx significantly decreased 30 minutes post exercise in the BFR condition with no change in the control condition. The researchers concluded that the decrease in bone resorption in the BFR condition might be due to the hypoxic and/or acidic environment caused by the restriction. Significant alterations in BTM's were also observed by Karabulut and colleagues (53) after six weeks of low-

intensity resistance training with BFR. 37 older male subjects were assigned to one of three groups: high-intensity (80% 1RM) resistance training, low-intensity (20% 1RM) resistance training with BFR, and non-exercise control. Both exercise groups performed the same upper and lower body exercises, but lower body (leg press and knee extensions) were performed for 3 sets by 8 reps at 80% 1RM for high-intensity group and 1 set for 30 reps, followed by 2 more sets at 15 reps at 20% 1RM for the BFR group. Cuff pressure was initiated at 160 mmHg at the onset of training and increased progressively throughout the intervention. Blood samples were obtained at baseline and at completion of the study in the morning following an overnight fast. Following the intervention, Bone ALP significantly increased by 20% in both exercise groups compared to the non-exercise control. CTx was unchanged in any group. The researchers concluded that low-intensity resistance training with BFR may be as effective as traditional high-intensity resistance training for improving bone health in older adults.

Currently, only one study on the effects of walking with BFR on bone markers has been published. Using the same protocol and participant population as the previously discussed walking study by Abe's group, Beekley and colleagues (10) measured the serum bone formation marker Bone ALP at baseline and three weeks after walk training with or without BFR. Blood samples were obtained after an overnight fast at approximately the same time in the morning with the post-training sample three days after the last exercise session. Following three weeks of training, a significant increase (10.8%) in Bone ALP was observed in the BFR walk group with no change in the control walk group.

Summary

From the literature available, it seems that high-impact weight-bearing exercises resulting in large GRF's and high-intensity resistance training provide premenopausal women with the most potential to increase bone mass prior to the menopause. However, these types of exercise may not be appropriate for women with already low bone mass at increased risk for fracture. Walking with BFR is known to result in increased thigh MCSA and lower-body strength in young men and older adults (1, 2) and has also been shown to increase levels of Bone ALP in college-aged men after three weeks (10). What is not known, however, is what effects walking with BFR have on serum markers of bone metabolism in young premenopausal women.

CHAPTER III

METHODOLOGY

The purpose of this study was to investigate the effects of 12 weeks of walking with and without blood flow restriction on metabolic markers of bone turnover in college-aged women. A secondary purpose of the study was to assess changes in thigh and calf MCSA, muscular strength, aerobic capacity, and cortical and trabecular bone characteristics of the tibia following the 12-week intervention.

Subjects

Seventy-six young women from the University of Oklahoma and city of Norman, OK and its surrounding area initially responded with interest to the study via contact from flyer posted in public or university-approved areas, direct recruitment from classrooms in the Department of Health and Exercise Science, and University mass email (Appendix A). Of the 76 respondents, 42 met the initial screening criteria checklist (Appendix B) and were scheduled for their first visit to the Bone Density Research Laboratory to fill out an Informed Consent (Appendix C) and questionnaires used to further determine study eligibility (Appendix D). Two additional subjects were excluded after the initial visit based on their answers to the health history questionnaire. Therefore, 40 women between the ages of 18-30 years satisfying the inclusion/exclusion criteria below were randomly assigned to one of the three study groups (WALK, BFR, and CON) and were scheduled for baseline testing. All methods and procedures were approved by the University of Oklahoma Health Sciences Center Institutional Review Board (IRB No. 16049) (Appendix E). Subject inclusion and exclusion criteria were as follows:

Inclusion Criteria

1. Women 18-30 years old.
2. Combined oral contraceptive use $\geq$ 1 month.
3. Free of chronic back or joint problems, cardiovascular disease, non-smokers, not pregnant, not taking antihypertensive drugs or any medication known to affect bone metabolism.
4. Not currently participating in exercise such as resistance training, circuit training, and/or moderate to high-intensity aerobic more than two days per week for the last 3 months.

Exclusion Criteria

1. Outside the 18-30 year age range.
2. Not taking combination oral contraceptives.
3. The use of any prescription medications other than combined oral contraceptives.
4. Weighing more than 300 lbs or over 74 inches (DXA machine requirement).
5. Structured exercise $\geq$ 3 days per week over the last 3 months.
6. Not able to perform the physical requirements of the study.
7. Pregnant women or women who think they may be pregnant.
8. Regular use of tobacco products (cigarettes, cigars, chew/snuff etc.).
9. Have a history of cardiovascular disease or thromboembolic disease.
10. Is a current student of Dr. Debra Bemben.
11. Is identified as a moderate- to-high risk individual as described by the American College of Sports Medicine:

a. At least two of the following: Father or brother, or mother or sister that has had a sudden death before 55 or 65 years of age, respectively; Is a current cigarette smoker or has quit smoking within the previous 6 months; Is on hypertensive medication or has a confirmed systolic or diastolic blood pressure ≥ 140 or 90 mmHg, respectively; Is on lipid lowering medication or has a total cholesterol level ≥200 mg/dL; Has a confirmed fasting blood glucose of ≥100 mg/dL; is clinically obese.

12. Having more than one risk factor for Thromboembolisms.
    a. Classified as Obese based on a Body Mass Index of ≥ 30;
    b. Diagnosed Crohn's or Inflammatory Bowel Disease;
    c. Past fracture of a hip, pelvis, or femur;
    d. Major Surgery within the last 6 months;
    e. Varicose veins are present;
    f. Family history of Deep Vein Thrombosis or Pulmonary Embolism.

Research Design

The current study was a randomized, controlled trial with repeated-measures design conducted at the University of Oklahoma's Bone Density Research and Neuromuscular laboratories housed in the Department of Health and Exercise Science. The duration of the study was 14 weeks, including one week for pre-testing, 12 weeks of training (4 times per week for a total of 48 sessions), and one week for post-testing. Upon the initial visit, subjects were informed about the study details and given ample time to ask questions. Subjects then filled out several questionnaires including an

informed consent form and other questionnaires used to determine study eligibility. Once determined eligible, 40 women aged 18-30 years from the University of Oklahoma and city of Norman, OK and its surrounding area were randomly assigned using a random number generator computer program to one of three groups: low-intensity treadmill walk group with blood flow restriction (BFR, n=14), low-intensity treadmill walk control group (WALK, n=14), or a non-exercise control group (CON, n=12) and scheduled for their next three testing visits. On the first testing day, subjects arrived at the university's health clinic to have their blood sampled after an overnight fast between 8:00 and 10:00am. After blood sampling, subjects came to the Bone Density Research laboratory to undergo a urine pregnancy test to ensure they were not pregnant prior to undergoing DXA and pQCT bone scans. After scans were completed, subjects were taken to the Neuromuscular laboratory and familiarized with the strength testing procedures and equipment. Day two of testing consisted of 1RM strength testing for knee extension and flexion and MVCs of the knee extensor/flexors at knee joint angles of 30 and 60 degrees. The third day of testing included a maximal graded exercise treadmill test for determination of walking speed used during the training intervention as well as VO_{2peak}. All measures previously described were performed at baseline and post-training in the same testing order. However, the post-training blood samples were obtained in the Bone Density Research laboratory by a nurse rather than the university's health center to accommodate a large number of subjects within a restricted time frame.

Exercise Protocol

Subjects in the BFR and WALK groups walked on a motor-driven treadmill four times per week for 12 weeks (48 total sessions) at a speed associated with 45% $VO_{2peak,}$ determined during their baseline maximal treadmill test. The treadmill speed was maintained throughout the duration of the study. However, the incline of the treadmill was increased by 1% at the start of week five and again at week nine. During the first week, each exercise session was 10 minutes in duration. At the start of week two, session duration was increased to 15 minutes and again increased to 20 minutes at the start of week three and maintained until the end of the study. Before each exercise session, the BFR group was fitted with pneumatic elastic pressure cuffs (5cm in width) (Kaatsu-Mini, Tokyo, Japan) around the most proximal portion of the thighs. Subjects were seated in a chair and the cuffs were applied with an initial sitting pressure of 50 mmHg. The cuffs were then inflated to 120 mmHg, held for 30 seconds and then deflated. This process was repeated by adding 10 mmHg pressure until the target exercise pressure was attained. Initial exercise pressure was set at 140 mmHg for weeks one through four, increased to 160 mmHg during weeks five through eight, and increased to 180 mmHg for the final four weeks. This pressure progression was tolerated well by all subjects with the exception of one, who visually had the smallest thigh circumference among the BFR group and was therefore maintained at 160 mmHg for the final four weeks of the intervention. This pressure stimulus was chosen based on pilot work performed in our laboratory which was well tolerated during four weeks of BFR walking. The cuff pressure was maintained by an electronic air pressure system throughout the exercise session and was released immediately on completion of the

session. Heart rate and rate of perceived exertion were monitored and recorded every two minutes during each exercise session. A table illustrating the exercise protocol is provided in the appendices (Appendix F). The CON group was asked not to change their current physical activity levels for the duration of the training period and only participated in the baseline and post-training testing sessions.

Questionnaires

After consent had been given, subjects completed a health history and status questionnaire, Physical Activity Readiness Questionnaire (PAR-Q), and menstrual history questionnaire. All three questionnaires were used to determine any additional exclusion criteria including menstrual status and oral contraceptive use. In addition, a 3-day dietary food log was completed for Thursday-Saturday of the first week of exercise and again during the last week of exercise (Appendix D). Food logs were analyzed using ESHA Food Processor version 10.8 (ESHA Research, INC., Salem, OR) for average daily caloric intake, protein intake, calcium intake, and vitamin D intake.

Body Composition/Bone Mineral Density

1. Dual Energy X-ray Absorptiometry (DXA)

All participants had DXA (GE Medical Systems, Lunar Prodigy encore software version 10.50.086, Madison, WI) scans to assess areal bone mineral density (aBMD) of the total body, dual proximal femur (femoral neck, trochanter, and total hip) and AP spine (L2-L4). A total body scan was performed to obtain bone free lean leg mass (LegLM), total bone free lean body mass (BFLBM), and percent body fat (%BF). Quality assurance testing (QA) was performed each day that scans were performed to ensure that the DXA was operating properly. The first step of QA for the DXA was a

scanning calibration block of known density, and a series of mechanical functioning tests, which the software ran automatically. All individual tests had to pass for the overall QA to pass. The second step of the QA testing was scanning a phantom spine block of known density. The L2-L4 density had to fall within the predetermined range to pass.

For the total body scan, the participants laid supine on the DXA table with arms close to the sides. Velcro straps were placed around their legs to ensure that the legs remained still and relaxed. For the AP lumbar spine scan, the legs were lifted and set on a foam block, such that the there was a bend in the knee, and the angle created by the thighs and the scanning bed was 45-90 degrees. The technician ensured that the iliac crests were even, and the lumbar spine was resting flat on the scanner bed. The scanner arm was centered with the torso as marked by the participant's navel, and placed approximately 5 cm below the navel to ensure that the iliac crests as well as the T12 vertebra were visible on the scan. The subjects' arms were crossed over the chest and then moved to directly over the chin and the scan was started. Scan speeds for the total body and lumbar spine were determined by the measured thickness of the subject at the naval (Thick = >25 cm; Standard = 13 – 25 cm; and Thin = < 13 cm). The dual femur scans were performed using the standard setting. Participants' legs were internally rotated and secured in place to ensure proper exposure of the femoral neck and the femur was positioned parallel to the scanning boundary. The scan began just below the pubic symphysis, centered on the thigh being scanned, and finished 3 to 4 sweeps above the head of the femur.

All scans were performed by a trained technician with day to day technician precision ranging from 0.38 – 1.1% for the sites of interest.

2. Peripheral Quantitative Computed Tomography (pQCT)

All participants had their total, trabecular and cortical volumetric bone mineral density (vBMD) and volumetric bone characteristics at 4%, 38% and 66% of the limb length proximal to the boney endplate of the right tibia, and 50% of the right femur determined by a peripheral quantitative computed tomography scanner, XCT 3000 with software version 6.00 (Stratec Medizintechnik GmbH, Pforzheim, Germany) by a single trained pQCT technician. Quality assurance test scans were completed each testing day, where a phantom cone of known densities underwent a scout view scan, and a series of 4 scans that the software ran automatically. The densities had to be within 99% accuracy in order for the quality assurance test to pass. Scans were acquired with a voxel size of 0.4 mm, slice thickness of 2.2 mm, and a scan speed of 20 mm/sec. Analysis of bone slices were performed using custom macros using the XCT software. Analysis thresholds were chosen to separate cortical bone from trabecular bone, and bone from fat and muscle. Thresholds used for the bone analyses at the 4% site were Contmode 3, Peelmode 4, Threshold1 169, Threshold 2 650, Cortmode 2, Threshcrt1 480, Threshcrt 0. Thresholds used for the tibia 38% and 66% and femur 50% sites were Contmode 1, Peelmode 2, Threshold1 710, Threshold2 710, Cortmode 2, and Threshcrt 710. An additional line of analysis with a threshold of 480 mg/cm^{3}was used for both the 38% and 66% tibia sites. This threshold allows subcortical bone to be included in the analysis for the determination of stress-strain index (SSI), a measure of bone torsional and bending strength. Muscle cross-sectional area (MCSA) of the tibia 66%

and femur 50% sites were determined by drawing a region of interest around the total CSA scan and analyzed for MCSA using two lines of threshold driven analyses along with the median smoothing filter F01F06U01. The first analysis separates fat + marrow from the total CSA leaving total bone + muscle area. In the second analysis, total bone area is determined and subtracted from the total bone + muscle area, effectively leaving MCSA only. In the Bone Density Research Laboratory, the in vivo precision for measurement of total vBMD, BMC and bone area is 0.3% - 1.9%. In vivo precision ranges for measuring vBMD, BMC and area variables for the trabecular and cortical compartments are 1.1% - 5% and 0.5% - 1.7%, respectively. In vivo precision for the femur MCSA measurement is 0.9%.

Strength Testing

1**.** One Repetition Maximum (1RM)

1RM testing was performed at baseline and post-training to determine dynamic muscular strength of the knee flexors and extensors using knee flexion and knee extension weight machines (Cybex International, Inc., Medway, MA.). Participants performed a 5-minute warm up on a stationary cycle ergometer followed by a warm up set of 8-10 repetitions using 50% of their predicted 1RM. After a one-minute rest, the load was increased and subjects performed a set of 3-5 repetitions. Following a two-minute rest, the load was increased and subjects performed their first maximal attempt. After each successful attempt through the full range of motion, the load was increased until a failed attempt occurred. Two-minute rest periods were given between each maximal attempt and 1RMs were achieved between 3-5 attempts.

2. Maximal Voluntary Contraction (MVC)

MVCs of the knee flexors and extensors were performed at baseline and post-training on an isokinetic dynamometer (Biodex Medical Systems, Shirley, NY) to determine maximal isometric strength. Knee flexion and extension were performed at joint angles of 30 and 60 degrees, as measured by a goniometer. Participants performed one repetition for each muscle group at 50% of maximal perceived effort. After this initial warm-up, participants performed three repetitions at maximal effort for the knee flexor and extensor muscles. One-minute rest periods were provided between warm-up repetitions and between maximal trials. The highest torque output measured for each of the three maximal attempts was considered the MVC for that particular test.

Aerobic Capacity

Participants underwent a maximal graded exercise treadmill test at baseline and post-training to determine their aerobic capacity. A mouthpiece attached to a head harness was secured to the subject and nose clips were placed on the subject's nose to allow only mouth breathing. Once the subject was ready to begin the test protocol, the subject was asked to straddle the treadmill with both legs while the treadmill was turned on to an initial warm up speed of 2.0 mph at a 0% grade. The subject carefully stepped onto the treadmill and walked at this initial warm up workload for 90 seconds. The treadmill speed was then increased to 3.0 mph and held there for an additional 90 seconds. The treadmill speed was then increased to 3.5 and 4.0 mph and held at each speed for 90 seconds. This warm up procedure was used to ensure the subject had adequate warm up time and also used to prescribe the exercise intensity for the training sessions. After warm up, the speed was increased to 5.5 mph and held constant for the remainder of the test. Every two minutes thereafter, the treadmill grade was increased

2%. The subject was encouraged to exercise to their volitional fatigue unless the subject experienced clinical signs to terminate the exercise test as stated by the ACSM's Guidelines for Exercise Testing and Prescription (i.e., angina, dyspnea, dizziness, a decline in systolic blood pressure, lightheadedness, confusion, ataxia, cyanosis, nausea, chronotropic impairment, failure of the monitoring system, or other signs or symptoms for terminating the test). VO_{2peak} was determined using the TrueOne 2400 metabolic measurement system (Parvo Medics, Sandy, UT).

Blood Sampling

Resting blood samples (approximately 7 ml) were collected by a nurse from the subject's antecubital vein via venipuncture between the hours of 8:00 and 10:00 am following an overnight fast at baseline and post-training. The post-training blood sample was collected 48-72 hours following the last exercise session. Blood samples were allowed to clot at room temperature for 30 minutes. Each sample was separated by centrifugation (IEC Centra CL3, Thermo Electron Corporation, Milford, MA) and serum transferred to eight polystyrene microtubes labeled by subject ID and date and stored in a -80°C freezer until assay analyses. Serum samples were only thawed one time prior to analysis.

Bone Turnover Marker Assays

1. Bone-specific Alkaline Phosphatase (Bone ALP):

Serum samples were measured in duplicate using the MicroVue Bone ALP EIA Kit (Quidel Corporation, San Diego, CA, U.S.A.). The EIA kit utilizes a monoclonal anti-BAP antibody. The catalytic activity of the captured enzyme is used to measure Bone ALP activity in serum. Enzyme activity is determined spectrophotometrically and

Bone ALP concentrations are then calculated from a calibration curve fit with a quadratic equation. The Bone ALP assay was performed precisely to manufacturer's specifications. Values are expressed as Units per Liter (U/L), with each unit representing one mole of p-nitrophenyl phosphate (pNPP) hydrolyzed per minute at 25°C. Intra-assay and inter-assay percent coefficient of variation were 6.7-9.5%, and 4.7%, respectively.

2. Tartrate-resistant acid phosphatase isoform 5b (TRAP5b)

Serum samples were analyzed in duplicate for TRAP5b using the MicroVue TRAP5b ELISA kit (Quidel Corporation, San Diego, CA). Enzyme activity is determined spectrophotometrically and the TRAP5b concentrations are then calculated from a calibration curve fit with a quadratic equation. The assay kit was performed precisely to manufacturer's specifications and guidelines. Units are reported in Units per Liter (U/L). Intra-assay and inter-assay percent coefficient of variation were 0.7-5.5% and 3.7%, respectively.

Data Analyses

Data are reported as mean ± SE for all dependent variables. Statistical analyses were performed using PASW for Windows version 18.0 (PASW, Inc., Chicago, IL). Group differences in baseline values for the dependent variables were determined using one-way analysis of variance (ANOVA). If significant group differences existed at baseline, a one-way analysis of covariance (ANCOVA) was used to determine group differences in the post variables using the baseline variable as the covariate. In the case that no group differences existed at baseline, a 3x2 mixed-factorial ANOVA with repeated measures ([group (CON vs. WALK vs. BFR) x time (pre vs. post)] was used to

analyze changes between groups and across time for each dependent variable. If a significant group x time interaction was detected, follow-up analyses included paired-samples t-tests and one-way ANOVAs. Percent change from baseline was calculated for the main dependent variables using the following equation: $\%\Delta = [(post - pre) / pre] X 100$. Normality of all percent change from baseline dependent variables was determined using a Kolmogorov-Smirnov procedure. Based on this analysis, eight of the percent change variables were not normally distributed. Therefore, Kruskal-Wallis one-way ANOVAs with pair-wise comparisons were used to determine group differences in these variables. For all other percent change variables, a one-way ANOVA was used to determine group differences.

Pearson Product Moment Correlation Coefficients were run to determine relationships between the bone turnover markers and baseline aBMD and dietary variables and between baseline dietary variables and the tibia bone variables. Significant correlates were then used as covariates for the bone turnover markers and tibia bone variables in the analysis described above. The level of significance was set at $p \leq 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

The purpose of this study was to investigate the effects of 12 weeks of walking with and without blood flow restriction on metabolic markers of bone turnover in college-aged women. A secondary purpose of the study was to assess changes in thigh and calf MCSA, muscular strength, aerobic capacity, and cortical and trabecular bone characteristics of the tibia following the 12-week intervention.

Subject Characteristics

A total of 40 subjects originally qualified for the study and were randomized into one of the three study groups: low-intensity treadmill walk group with blood flow restriction (BFR, n=14), low-intensity treadmill walk control (WALK, n=14), or a non-exercise control group (CON, n=12). However, nine subjects withdrew or were withdrawn from the study for the following reasons: five due to time commitments, one stopped the use of combined oral contraceptives, one lost employment and relocated to another city, one became ill with mononucleosis, and one was lost to post-testing follow-up. Therefore, 31 subjects (BFR, n=11; WALK, n=10, CON, n=10) completed the entire study protocol with a 100% attendance rate for the 48 exercise sessions.

Baseline physical characteristics and average daily nutrient intake variables estimated from a 3-day food log are presented in Table 1. No significant group differences existed at baseline.

Table 1. Baseline Subject Characteristics

	Group		
Variable	BFR (n=11)	WALK (n=10)	CON (n=10)
Age (yrs)	20.5 ± 0.6	21.5 ± 0.7	20.9 ± 0.6
Height (cm)	166.3 ± 1.8	166.6 ± 1.8	165.8 ± 1.7
Weight (kg)	58.4 ± 2.2	63.9 ± 2.3	61.9 ± 2.2
BMI (kg/m^2)	21.1 ± 0.7	23.1 ± 0.8	22.5 ± 0.5
% Body Fat	29.5 ± 1.5	33.6 ± 2.7	32.7 ± 1.7
Dietary Intake:			
Total CI (kcal/day)	2157.8 ± 148.1	1973.5 ± 213.7	1892 ± 193.1
Ca^{2+} (mg/day)	600.7 ± 86.1	694.1 ± 96.5	829.6 ± 169.1
Vitamin D (IU's/day)	53.4 ± 18.9	35.0 ± 19.5	66.8 ± 20.4

Values are mean ± SE. BFR: Blood flow restriction while walking, WALK: Walk only, CON: Non-exercise control, BMI: Body Mass Index, CI: Caloric Intake.

Areal Bone Mineral Density

Group values for aBMD of the total body (TB), hip, and spine at baseline and post-training are presented in Table 2. Baseline aBMD of the AP lumbar spine (L2-L4) was used to describe bone health status of each group using their associated Z-scores for age, gender, and ethnicity using the ISCD classification for premenopausal females (6). Under this classification, any female with a Z-score of $\leq$ -2.0 is considered to have low aBMD for the spine. Only one subject in the WALK group met the ISCD criterion for low aBMD for the spine. All other subjects had a spine Z-score > -2.0. Significant group differences at baseline were found for TB aBMD and femoral neck aBMD. One way ANCOVA adjusting for baseline differences did not detect significant group differences in post-training TB aBMD or femoral neck aBMD. Similarly, no main effects for time or group x time interactions were found for total hip or L2-L4 aBMD. However, mixed factorial ANOVA with repeated measures detected a significant

($p<0.01$) group x time interaction for trochanter aBMD. Follow up analyses revealed that the CON group significantly ($p<0.01$) increased trochanter aBMD from baseline.

Table 2. aBMD of the Total Body, Hip, and Spine Before and After Training.

	Group					
	BFR (n=11)		WALK (n=10)		CON (n=10)	
Variable	Pre	Post	Pre	Post	Pre	Post
TB aBMD	1.064 ± 0.018	1.071 ± 0.021	1.117 ± 0.024	1.115 ± 0.023	1.163 ± 0.028	1.165 ± 0.027
Fem Neck aBMD	0.990 ± 0.027	0.985 ± 0.025	1.017 ± 0.033	1.022 ± 0.035	1.095 ± 0.024	1.098 ± 0.025
Troch aBMD †	0.771 ± 0.020	0.772 ± 0.020	0.807 ± 0.044	0.805 ± 0.045	0.852 ± 0.020	0.863 ± 0.020*
Tot Hip aBMD	0.983 ± 0.022	0.983 ± 0.022	1.018 ± 0.042	1.018 ± 0.044	1.087 ± 0.024	1.094 ± 0.024
L2-L4 aBMD	1.185 ± 0.036	1.182 ± 0.035	1.181 ± 0.034	1.185 ± 0.031	1.292 ± 0.045	1.286 ± 0.049

Values are mean ± SE. aBMD: areal Bone Mineral Density, TB: Total Body, Fem: Femoral, Troch: Trochanter, Tot: Total, BFR: Blood Flow Restriction, WALK: Walking only, CON: Non-exercise Control. aBMD variables expressed in g/cm^2.
† Significant group x time interaction, p=0.008. * Significant increase from Pre, p=0.009.

Serum Markers of Bone Turnover

Resting serum concentrations of Bone ALP and TRAP5b at baseline and post-training for each group are presented in Table 3. There were no significant group differences at baseline for either bone marker. A significant (p=0.002) main effect for time and significant (p=0.02) group x time interaction was detected for Bone ALP. Follow up analyses revealed significant reductions in serum Bone ALP concentrations in both BFR (p=0.02) and CON (p=0.02) groups after the 12-week intervention. No significant main effects for time or group x time interactions existed for serum TRAP5b, and this remained true after adjusting for TB aBMD using ANCOVA. No significant main effects for time or group x time interactions existed for Bone ALP to TRAP5b ratio. Reference serum values provided by the ELISA kit manufacturer for premenopausal women 25-44 years of age are 11.6-29.6 U/L and 1.5-4.3 U/L for Bone ALP and TRAP5b, respectively. Several participants had baseline Bone ALP values outside the normal reference range. Specifically, 30% (3/10), 55% (6/11), and 70% (7/10) of participants in the WALK, BFR, and CON groups, respectively, had baseline

values higher than the reference range. This is not unexpected, as the women in this study are younger than the established reference age range. It has been previously shown that women below 25 years of age have higher rates of bone turnover, likely due to continued accretion of bone mass (40). All participants were within the normal reference range for serum concentrations of TRAP5b.

Table 3. Serum Markers of Bone Turnover Before and After Training.

	Group					
	BFR (n=11)		WALK (n=10)		CON (n=10)	
Variable	Pre	Post	Pre	Post	Pre	Post
Bone ALP (U/L)†	29.6 ± 2.8	27.6 ± 2.4*	28.0 ± 2.4	28.4 ± 2.2	34.7 ± 3.4	31.1 ± 3.2*
TRAP5b (U/L)	2.8 ± 0.3	2.7 ± 0.4	2.6 ± 0.3	2.6 ± 0.3	2.5 ± 0.1	2.5 ± 0.2
Ratio	11.0 ± 1.1	11.2 ± 1.5	11.5 ± 1.3	11.5 ± 0.9	14.2 ± 0.3	12.8 ± 1.4

Values are mean ± SE. Bone ALP: Bone-specific Alkaline Phosphatase, TRAP5b: Tartrate-Resistant Acid Phosphatase Isoform 5b, BFR: Blood Flow Restriction with walking, WALK: Walking only, CON: Non-exercise Control. † p=0.02, significant group x time interaction , * p=0.02, significant decrease from Pre

Percent changes from baseline in serum concentrations of Bone ALP, TRAP5b, and the ratio of Bone ALP to TRAP5b are shown in Figure 1. One-way ANOVA detected significant group differences in percent changes of serum Bone ALP concentrations from baseline (p=0.005). Compared to WALK (1.9%), the CON (-9.7%) group experienced a significant (p=0.004) reduction from baseline in serum Bone ALP concentration. No significant group differences in percent change from baseline were detected for serum TRAP5b or ratio of Bone ALP to TRAP5b.

The bone marker responses were not correlated with percent changes in body weight or total caloric intake. However, baseline vitamin D intake was positively related to the percent change in TRAP5b (r=0.37, p=0.04).

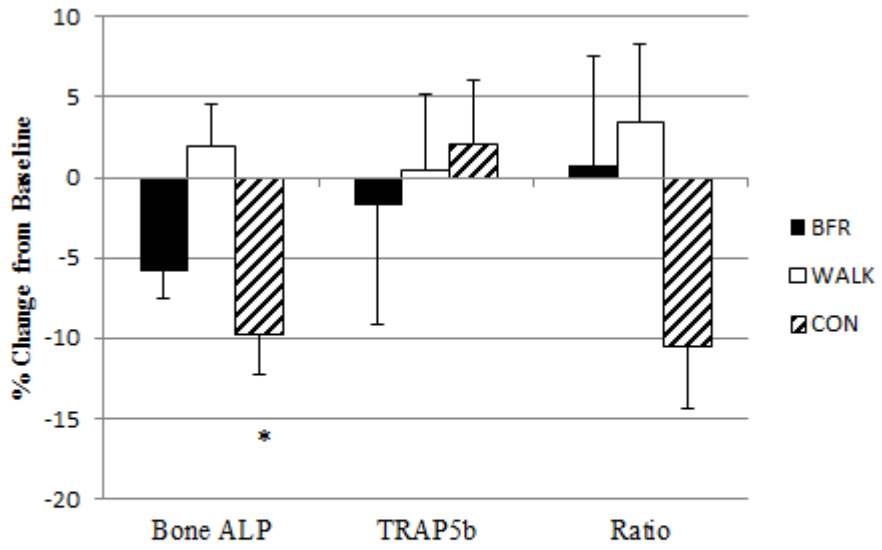


Figure 1. Percent Change in Bone Turnover Markers After Training.
Values are mean ± SE. Bone ALP: Bone-specific Alkaline Phosphatase, TRAP5b: Tartrate-Resistant Acid Phosphatase Isoform 5b. * $p<0.01$, significantly different from WALK group

Body Composition

Baseline and post-training measurements of body composition for each group are presented in Table 4. There were no significant group differences at baseline for any of the variables. A significant group x time interaction was detected for body weight ($p=0.034$). However, follow-up analyses did not detect any significant post-training group differences or within-group differences over time. Figure 2 depicts the change in body weight for each group over the 12 week intervention. Percent change in body weight was significantly different between the two exercise groups ($p<0.05$) as the WALK had a 2.4 ± 1.3% increase in weight compared to a -0.8 ± 0.6% decrease in the BFR group. The CON group remained stable with a -0.07 ± 0.7% change in body weight. A significant main effect for time ($p=0.02$) and significant group x time interaction ($p=0.002$) was detected for MCSA at the tibia 66% site. Follow-up analyses

revealed that tibia 66% MCSA significantly increased from baseline in both BFR ($p<0.05$) and WALK ($p=0.002$) groups. No significant main effects for time or significant group x time interactions existed for percent body fat (% BF), bone free lean body mass (BFLBM), bone free lean leg mass (LegLM), or MCSA at the femur 50% site.

Table 4. Measures of Body Composition Before and After Training.

		Group		
Variable		BFR (n=11)	WALK (n=10)	CON (n=10)
Body Weight (kg) †	Pre	58.4 ± 2.2	64.0 ± 2.4	61.9 ± 2.2
	Post	58.0 ± 2.2	65.4 ± 2.4	61.9 ± 2.4
% Body Fat	Pre	29.5 ± 1.7	33.7 ± 2.7	32.7 ± 1.8
	Post	29.2 ± 1.5	34.8 ± 2.3	33.6 ± 1.9
BFLBM (kg)	Pre	36.8 ± 1.0	38.3 ± 1.0	37.8 ± 1.2
	Post	37.1 ± 1.1	38.8 ± 1.1	37.3 ± 1.2
Leg LM (kg)	Pre	12.9 ± 0.4	13.3 ± 0.4	13.2 ± 0.5
	Post	12.9 ± 0.4	13.7 ± 0.4	12.8 ± 0.5
Tib66% MCSA (mm^2) †	Pre	6134.5 ± 169.6	6333.4 ± 143.1	6309.3 ± 151.8
	Post	6241.4 ± 158.1*	6560.8 ± 165.6*	6216.8 ± 153.3
Fem50% MCSA (mm^2)	Pre	10733.4 ± 418.8	10930.1 ± 364.5	10982.3 ± 364.5
	Post	10676.9 ± 359.3	10929.3 ± 395.0	10686.7 ± 409.4

Values are mean ± SE. BFLBM: Bone Free Lean Body Mass, FM: Fat Mass, LM: Lean Mass, MCSA: Muscle Cross-Sectional Area, Tib66%: Tibia 66% site, Fem50%: Femur 50% site.
BFR: Blood Flow Restriction, WALK: Walking only, CON: Non-exercise Control.
† Significant group x time interaction. *Significant increase from Pre.

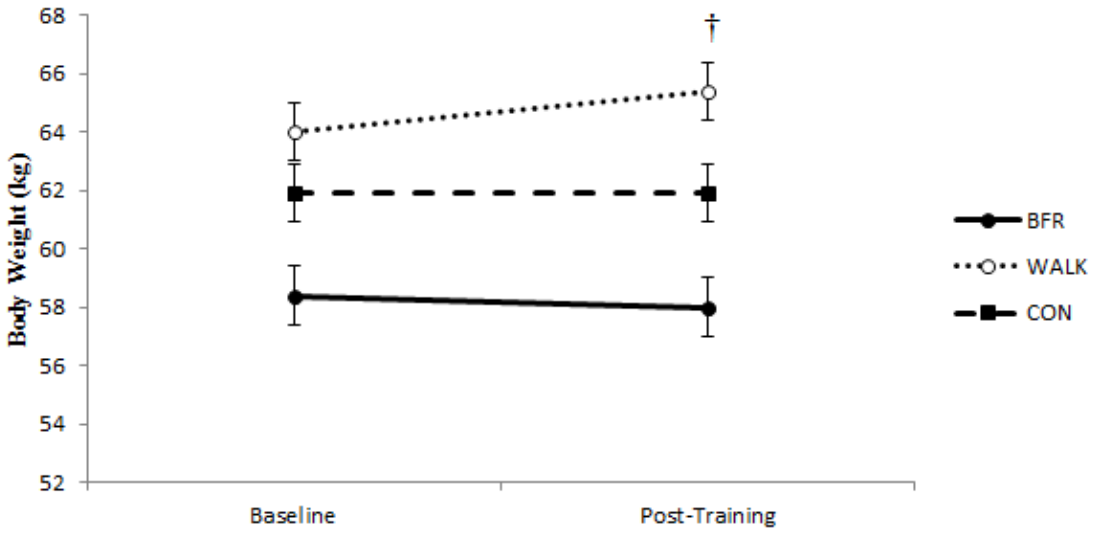


Figure 2. Change in Body Weight after 12 Weeks of Training.
Values are mean ± SE. † $p<0.05$, Significant group x time interaction.

Percent changes from baseline for body composition variables are shown in Figure 3. Significant group differences in percent changes from baseline were found for Tib66% MCSA ($p=0.002$). Compared to CON, WALK ($p=0.002$) and BFR ($p=0.042$) groups experienced a significant percent increase (CON: -1.5%, WALK: 3.6%, BFR: 1.8%) in Tib66% MCSA from baseline. No significant group differences in percent changes from baseline were detected for any other body composition variables.

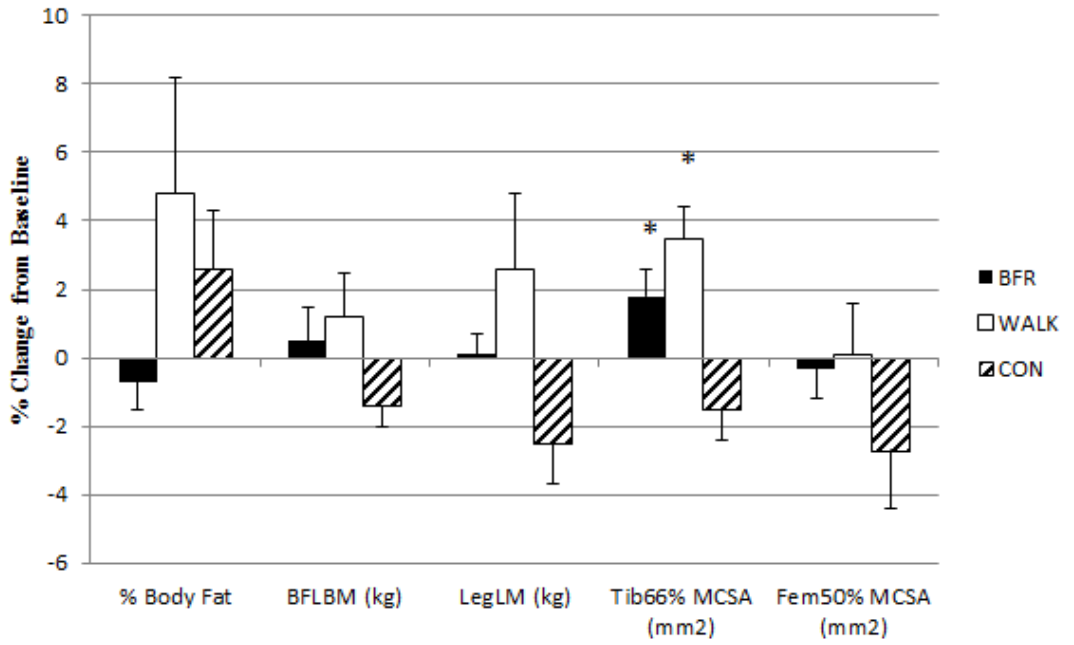


Figure 3. Percent Change in Body Composition Variables from Baseline.
Values are mean ± SE. BFLBM: Bone Free Lean Body Mass, LegLM:Leg Lean Mass, MCSA: Muscle Cross-Sectional Area, Tib66%: Tibia 66% site, Fem50%: Femur 50% site.
*Significantly Different ($p<0.05$) from CON group.

Muscle Strength

The 1RM and MVC muscle strength for the knee flexors and extensors at baseline and post-training are shown in Table 5. No significant group differences in strength existed at baseline. Repeated-measures ANOVA detected a significant main effect for time for MVC strength of the knee extensors at joint angles of 30 degrees ($p=0.02$) and 60 degrees ($p=0.004$), with no differences between groups. No significant differences for main effects or group x time interactions existed for MVC strength of the knee flexors at either joint angle. A significant group x time interaction was detected for 1RM knee extension strength ($p=0.014$). Follow-up analyses revealed a significant ($p=0.026$) increase in 1RM knee extension strength over time for the BFR

group (Figure 4). No significant main effects or group x time interactions existed for 1RM knee flexion strength.

Table 5. Muscle Strength Before and After Training.

		Group		
Variable		BFR (n=11)	WALK (n=10)	CON (n=10)
MVCs:				
Knee Ext 30° (N-m) +	Pre	116.3 ± 5.8	118.5 ± 9.0	123.1 ± 7.8
	Post	127.7 ± 7.4	128.2 ± 9.0	123.0 ± 9.0
Knee Flx 30° (N-m)	Pre	74.7 ± 4.1	84.9 ± 4.8	80.0 ± 5.5
	Post	78.8 ± 4.3	85.8 ± 2.8	80.6 ± 5.9
Knee Ext 60° (N-m)+	Pre	171.9 ± 9.9	174.8 ± 11.5	175.0 ± 9.5
	Post	187.3 ± 10.3	184.3 ± 11.1	180.2 ± 9.9
Knee Flx 60° (N-m)	Pre	73.3 ± 4.0	80.8 ± 4.0	75.3 ± 4.5
	Post	76.3 ± 4.0	77.4 ± 3.2	76.6 ± 5.0
1RM:				
Knee Extension (kg)†	Pre	57.3 ± 2.6	64.2 ± 2.4	61.4 ± 3.4
	Post	60.2 ± 3.4*	65.7 ± 2.4	60.0 ± 3.0
Knee Flexion (kg)	Pre	58.1 ± 2.2	61.4 ± 2.4	58.0 ± 2.9
	Post	60.7 ± 2.0	61.7 ± 2.5	57.7 ± 3.0

Values are mean ± SE. MVC: Maximal Voluntary Contraction, Ext: Extension, Flx: Flexion, 1RM: 1-Repetition Maximum, BFR: Blood Flow Restriction, WALK: Walking only, CON: Non-exercise Control.

† Significant group x time interaction. +Significant time effect.*Significant increase from Pre

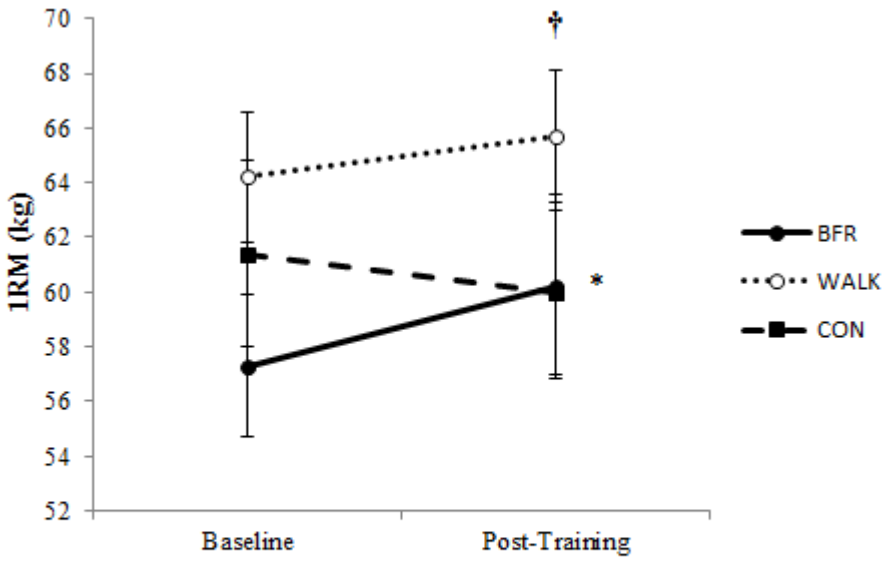


Figure 4. Change in 1RM Knee Extension Strenth After Training. Values are mean ± SE. 1RM: 1-Repetition Maximum. † $p<0.02$, significant group x time interaction. * $p<0.05$, significant increase over time (BFR).

Percent changes in knee extension and flexion 1RM muscle strength from baseline are shown in Figure 5. One-way ANOVA failed to detect significant group differences in percent changes from baseline in any of the MVC strength variables. However, a significant ($p=0.034$) group difference in percent change from baseline was detected for 1RM knee extension strength using the Kruskal-Wallis analysis. Follow-up analyses with pairwise comparisons revealed that BFR and CON groups were significantly ($p=0.028$) different from each other. No group differences were observed for percent change from baseline in 1RM knee flexion strength.

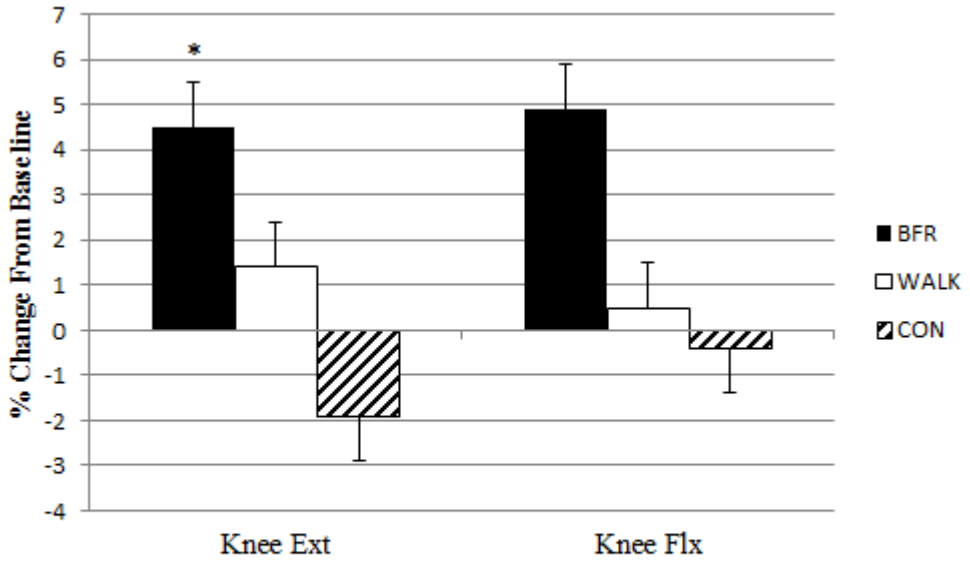


Figure 5. 1RM Knee Extension and Flexion Strength Percent Change from Baseline
Values are mean ± SE. 1RM: 1-Repetition Maximum, Ext: Extension, Flx: Flexion
* p=0.02, Significantly different from CON.

Aerobic Capacity

VO_{2peak} at baseline and post-training for each group is presented in Table 6. It should be noted that one subject in the CON group did not complete the baseline maximal graded treadmill test due to not being comfortable only breathing through her mouth during the test. Therefore, only data for 9 subjects (CON, n=9) were used in the final analysis. No significant group differences inVO_{2peak} existed at baseline. Repeated-measures ANOVA failed to detect any significant main effects for time or group x time interactions for VO_{2peak}.

Table 6. VO_{2peak} Before and After Training.

		Group		
Variable		BFR (n=11)	WALK (n=10)	CON (n=9)
VO_{2peak} (ml/kg/min)	Pre	39.2 ± 1.0	38.3 ± 1.9	37.6 ± 1.3
	Post	39.3 ± 1.4	37.2 ± 1.4	35.2 ± 1.9

Values are mean ± SE. BFR: Blood Flow Restriction, WALK: Walking only, CON: Non-exercise Control.

Group percent changes in VO_{2peak} from baseline are depicted in Figure 6. One-way ANOVA failed to detect any significant group differences in percent change of $VO2_{peak}$ from baseline.

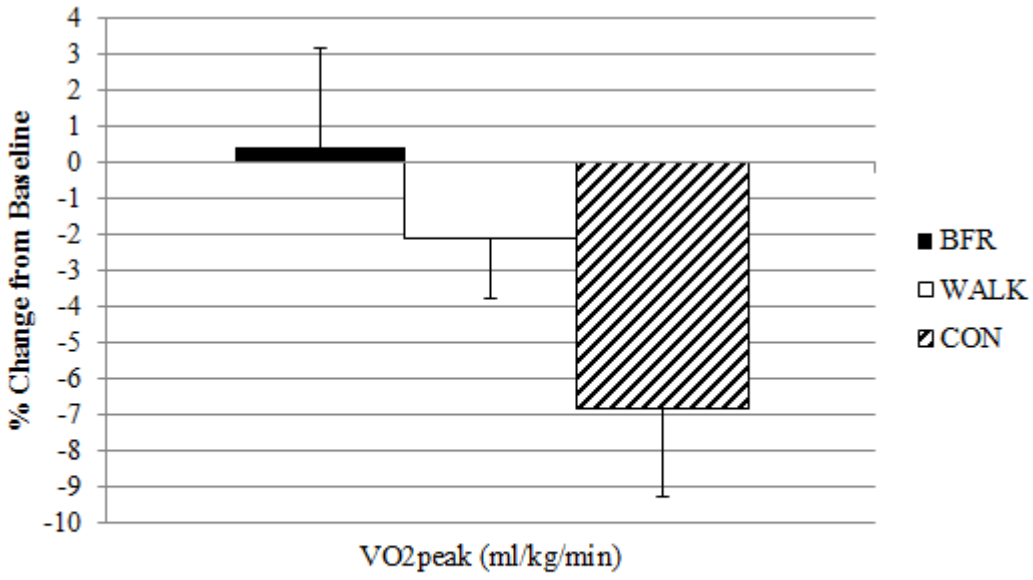


Figure 6. VO_{2peak} Percent Change from Baseline.
Values are mean ± SE.

Bone Characteristics of the Tibia

Bone characteristics of the Tibia 4% site at baseline and post-training for each group are presented in Table 7. Cortical bone measurements were not analyzed for this site, as the distal tibia is composed primarily of trabecular bone. Therefore, only total and trabecular bone area, content, and volumetric bone mineral density (vBMD) are reported. Baseline vitamin D intake was negatively correlated ($r=-0.35$ to -0.53, $p<0.05$) to several of the tibia bone variables, and was therefore used as a covariate. No significant group differences at baseline existed among any of the tibia 4% site variables. No significant group x time interaction effects were detected for any of the tibia 4% variables, and this remained true after adjusting for vitamin D intake.

However, a significant main effect for time was found for trabecular bone content (p=0.036) and trabecular vBMD (p=0.024), both of which decreased over the study duration. Percent changes from baseline in the tibia 4% site bone variables for each group are presented in Table 10. No significant differences in percent change from baseline were detected for any of the tibia 4% bone variables.

Table 7. Bone Characteristics at the 4% Tibia Site Before and After Training.

		Group		
Variable		BFR (n=11)	WALK (n=10)	CON (n=10)
Tibia 4%				
Tot Bone Content (mg/mm)	Pre	334.6 ± 17.8	364.1 ± 28.1	349.3 ± 19.9
	Post	315.6 ± 16.5	360.5 ± 25.5	331.6 ± 14.5
Tot vBMD (mg/cm^3)	Pre	305.666 ± 18.620	316.490 ± 17.597	315.360 ± 11.171
	Post	295.773 ± 10.217	305.490 ± 19.777	304.150 ± 9.613
Tot Bone Area (mm^2)	Pre	1101.50 ± 30.16	1151.31 ± 61.38	1109.41 ± 50.76
	Post	1065.40 ± 33.94	1183.92 ± 43.33	1096.34 ± 47.81
Trab Bone Content (mg/mm) +	Pre	263.2 ± 13.9	290.8 ± 22.1	279.5 ± 19.7
	Post	249.3 ± 15.8	285.6 ± 19.9	260.8 ± 14.1
Trab vBMD (mg/cm^3) +	Pre	275.618 ± 17.567	291.260 ± 16.718	285.340 ± 11.173
	Post	264.836 ± 11.617	277.610 ± 17.977	271.270 ± 7.765
Trab Area (mm^2)	Pre	964.79 ± 33.51	1004.48 ± 58.80	976.40 ± 48.36
	Post	937.85 ± 30.19	1034.85 ± 43.74	963.68 ± 46.01

Values are mean ± SE. Tot: Total, Trab: Trabecular, vBMD: Volumetric Bone Mineral Density.
BFR: Blood Flow Restriction, WALK: Walking only, CON: Non-exercise Control.+ Significant time effect.

Bone characteristics of the tibia 38% and 66% sites at baseline and post-training for each group are presented in Tables 8 and 9, respectively. Trabecular bone characteristics were not analyzed for the tibia 38% and 66% sites, as these sites are composed primarily of cortical bone tissue. Therefore, only total and cortical bone content, vBMD, and area as well as cortical shell thickness, periosteal and endosteal circumference, and strength strain index (SSI) are reported. There were no significant baseline group differences for any of the tibia 38% and 66% variables. No significant

group x time interaction effects were detected for any of the tibia 38% and 66% variables, and this remained true after adjusting for baseline vitamin D intake. However, significant time effects were found for total bone content ($p=0.036$) and SSI ($p=0.011$) at the 38% site as well as total bone content ($p=0.043$), total vBMD ($p=0.029$), total bone area ($p=0.001$), periosteal circumference ($p=0.002$), and endosteal circumference at the 66% site. Total vBMD at the 66% site decreased post-training, whereas the other variables with significant time effects increased over the study duration. Percent changes from baseline in the tibia 38% and 66% site bone variables for each group are presented in Table 10. No significant differences in percent change from baseline were detected for any of the tibia 38% or 66% bone variables.

Table 8. Bone Characteristics at the 38% Tibia Site Before and After Training.

		Group		
Variable		BFR (n=11)	WALK (n=10)	CON (n=10)
Tibia 38%				
Tot Bone Content (mg/mm) +	Pre	292.3 ± 8.1	320.7 ± 12.9	312.6 ± 8.4
	Post	294.6 ± 7.4	323.9 ± 11.3	314.0 ± 8.8
Tot vBMD (mg/cm^3)	Pre	911.800 ± 14.075	916.140 ± 25.319	923.880 ± 10.619
	Post	914.218 ± 13.666	924.140 ± 21.842	925.640 ± 9.586
Tot Bone Area (mm^2)	Pre	321.27 ± 10.12	350.05 ± 10.50	338.88 ± 10.49
	Post	322.98 ± 9.23	350.93 ± 10.43	339.70 ± 10.53
Cort Bone Content (mg/mm)	Pre	278.2 ± 7.8	307.8 ± 12.3	300.4 ± 8.3
	Post	280.5 ± 6.9	310.9 ± 10.5	301.7 ± 8.6
Cort vBMD (mg/cm^3)	Pre	1183.418 ± 5.579	1197.300 ± 6.362	1185.720 ± 6.823
	Post	1185.218 ± 5.853	1197.360 ± 5.929	1187.550 ± 4.448
Cort Area (mm^2)	Pre	235.35 ± 7.44	257.25 ± 10.68	253.41 ± 6.91
	Post	236.87 ± 6.51	259.88 ± 9.33	254.05 ± 7.04
Cort Thickness (mm)	Pre	4.90 ± 0.13	5.16 ± 0.23	5.18 ± 0.09
	Post	4.92 ± 0.13	5.21 ± 0.20	5.18 ± 0.09
Periosteal Circ (mm)	Pre	63.46 ± 1.02	66.26 ± 0.99	65.19 ± 1.00
	Post	63.64 ± 0.92	66.34 ± 0.97	65.27 ± 1.00
Endosteal Circ (mm)	Pre	32.67 ± 1.12	33.85 ± 1.46	32.66 ± 0.92
	Post	32.70 ± 1.14	33.54 ± 1.45	32.70 ± 0.89
SSI (mm^3) +	Pre	1282.28 ± 54.34	1480.31 ± 68.94	1403.43 ± 65.51
	Post	1301.87 ± 48.90	1484.32 ± 70.51	1416.59 ± 62.47

Values are mean ± SE. Tot: Total, Cort: Cortical, vBMD: Volumetric Bone Mineral Density, Circ: Circumference.
BFR: Blood Flow Restriction, WALK: Walking only, CON: Non-exercise Control.+ $p<0.05$, Significant time effect.

Table 9. Bone Characteristics at the 66% Tibia Site Before and After Training.

Variable		Group		
		BFR (n=11)	WALK (n=10)	CON (n=10)
Tibia 66%				
Tot Bone Content (mg/mm) +	Pre	337.3 ± 9.1	364.2 ± 11.1	358.7 ± 11.8
	Post	339.5 ± 8.9	364.6 ± 10.8	359.5 ± 11.7
Tot vBMD (mg/cm^3) +	Pre	714.491 ± 14.625	755.460 ± 26.658	728.050 ± 14.100
	Post	708.364 ± 14.517	752.730 ± 25.796	723.580 ± 13.724
Tot Bone Area (mm^2) +	Pre	473.56 ± 14.21	486.46 ± 18.99	494.34 ± 18.70
	Post	480.48 ± 13.41	488.40 ± 18.40	498.80 ± 19.69
Cort Bone Content (mg/mm)	Pre	309.2 ± 8.5	336.5 ± 10.9	329.9 ± 10.9
	Post	310.2 ± 8.5	336.5 ± 10.6	329.7 ± 10.4
Cort vBMD (mg/cm^3)	Pre	1157.282 ± 6.346	1170.660 ± 3.723	1155.800 ± 5.821
	Post	1159.173 ± 5.372	1170.310 ± 3.931	1151.130 ± 6.087
Cort Area (mm^2)	Pre	267.26 ± 7.63	287.41 ± 9.21	285.36 ± 8.96
	Post	267.74 ± 7.60	287.55 ± 9.04	286.37 ± 8.61
Cort Thickness (mm)	Pre	4.19 ± 0.11	4.53 ± 0.17	4.40 ± 0.10
	Post	4.15 ± 0.11	4.52 ± 0.17	4.39 ± 0.10
Periosteal Circ (mm) +	Pre	77.05 ± 1.17	78.05 ± 1.55	78.69 ± 1.47
	Post	77.63 ± 1.09	78.21 ± 1.50	79.04 ± 1.54
Endosteal Circ (mm) +	Pre	50.73 ± 1.36	49.56 ± 2.23	51.03 ± 1.55
	Post	51.56 ± 1.24	49.81 ± 2.18	51.03 ± 1.55
SSI (mm^3)	Pre	1999.00 ± 73.65	2136.94 ± 100.98	2162.79 ± 111.88
	Post	2029.62 ± 71.25	2148.82 ± 104.04	2162.27 ± 119.56

Values are mean ± SE. Tot: Total, Cort: Cortical, vBMD: Volumetric Bone Mineral Density, Circ: Circumference. BFR: Blood Flow Restriction, WALK: Walking only, CON: Non-exercise Control.+ $p<0.05$, Significant time effect.

Table 10. Percent changes in Tibia 4%, 38%, and 66% Site Bone Characteristics.

	Group								
	BFR			WALK			CON		
Variable	4%	38%	66%	4%	38%	66%	4%	38%	66%
Tot Bone Content	-4.7 ± 3.3	0.9 ± 0.5	0.7 ± 0.3	0.2 ± 3.8	1.3 ± 1.2	0.1 ± 0.2	-4.2 ± 2.4	0.4 ± 0.3	0.3 ± 0.3
Tot vBMD	-1.7 ± 3.0	0.3 ± 0.3	-0.8 ± 0.7	-3.7 ± 1.9	1.0 ± 0.9	-0.3 ± 0.2	-3.2 ± 2.0	0.2 ± 0.3	-0.6 ± 0.3
Tot Bone Area	-3.3 ± 1.2	0.6 ± 0.6	1.5 ± 0.7	4.8 ± 5.4	0.3 ± 0.2	0.5 ± 0.3	-1.0 ± 0.6	0.3 ± 0.2	0.9 ± 0.4
Trab Bone Content	-5.1 ± 3.2			-0.5 ± 4.2			-5.4 ± 2.9		
Trab vBMD	-2.5 ± 3.1			-4.8 ± 2.2			-4.3 ± 2.5		
Trab Area	-2.7 ± 1.0			5.3 ± 6.5			-1.2 ± 0.6		
Cort Content		1.0 ± 0.5	0.4 ± 0.5		1.3 ± 1.3	0.0 ± 0.3		0.4 ± 0.4	0.0 ± 0.4
Cort vBMD		0.2 ± 0.4	0.2 ± 0.2		0.0 ± 0.1	0.0 ± 0.1		0.2 ± 0.3	-0.4 ± 0.3
Cort Area		0.8 ± 0.8	0.2 ± 0.5		1.3 ± 1.3	0.1 ± 0.2		0.2 ± 0.3	0.4 ± 0.3
Crt Thickness		0.6 ± 0.6	-0.9 ± 0.9		1.6 ± 1.5	-0.3 ± 0.2		0.1 ± 0.2	-0.2 ± 0.3
Periosteal Circ		0.3 ± 0.3	0.8 ± 0.3		0.1 ± 0.1	0.2 ± 0.1		0.1 ± 0.1	0.4 ± 0.2
Endosteal Circ		0.1 ± 0.3	1.7 ± 0.9		-0.8 ± 0.8	0.6 ± 0.3		0.2 ± 0.2	0.7 ± 0.3
SSI		1.8 ± 0.9	1.7 ± 0.9		0.3 ± 0.4	0.5 ± 0.3		1.1 ± 0.6	-0.1 ± 0.8

Values are mean ± SE. Tot: Total, Cort: Cortical, vBMD: Volumetric Bone Mineral Density, Circ: Circumference, Trab: Trabecular, BFR: Blood Flow Restriction, WALK: Walking only, CON: Non-exercise Control.

Dietary Analysis

Average daily total caloric intake, protein intake, calcium intake, and vitamin D intake for week one and week twelve were determined using three-day dietary food logs. Average daily nutrient intake data for weeks 1 and 12 for each group are presented in Table 11. No significant group differences in any of the nutrient intake variables existed at week one. No significant group x time interaction effects were detected for any of the nutrient intake variables. However, a significant (p=0.008) time effect was found for average daily total calorie intake, where daily caloric intake decreased over the study duration. Figure 7 depicts the group changes in average daily total caloric intake from week 1 to week 12. There was a significant group effect for percent change in total caloric intake (p=0.034) with BFR and WALK being significantly different (BFR: -20.9 ± 4.5% vs. WALK: 2.3 ± 6.8%). The CON group only showed a slight

percent decrease in total caloric intake post-training (-5.8 ± 6.9%). All groups had an average daily intake of calcium and vitamin D during week 1 and week 12 below the average recommended daily allowance of 1000 mg/day and 600 IU/day, respectively, set forth by the Institutes of Medicine (96). However, these estimates are based on intake from the diet only. Vitamin supplementation was not documented by the three-day food log and sun exposures were not accounted for.

Table 11. Average Daily Nutrient Intake during Week 1 and 12.

		Group		
Variable		BFR (n=11)	WALK (n=10)	CON (n=9)
Total Calories (kcal)+	Pre	2157.8 ± 148.1	1973.5 ± 213.7	1892.9 ± 135.1
	Post	1688.7 ± 138.9	1939.4 ± 178.9	1714.7 ± 85.3
Total Protein (g)	Pre	73.5 ± 7.9	69.2 ± 6.3	72.9 ± 5.8
	Post	64.5 ± 4.7	64.8 ± 6.4	67.7 ± 5.1
Calcium (mg)	Pre	600.7 ± 86.1	694.1± 96.5	829.6 ± 169.1
	Post	530.7 ± 50.2	612.1± 96.5	606.5 ± 97.6
Vitamin D (IU)	Pre	53.4 ± 18.9	35.0 ± 19.5	66.8 ± 20.4
	Post	31.6 ± 12.5	41.1 ± 16.6	75.3 ± 28.7

Values are mean ± SE. BFR: Blood Flow Restriction, WALK: Walking only, CON: Non-exercise Control. + Significant time effect.

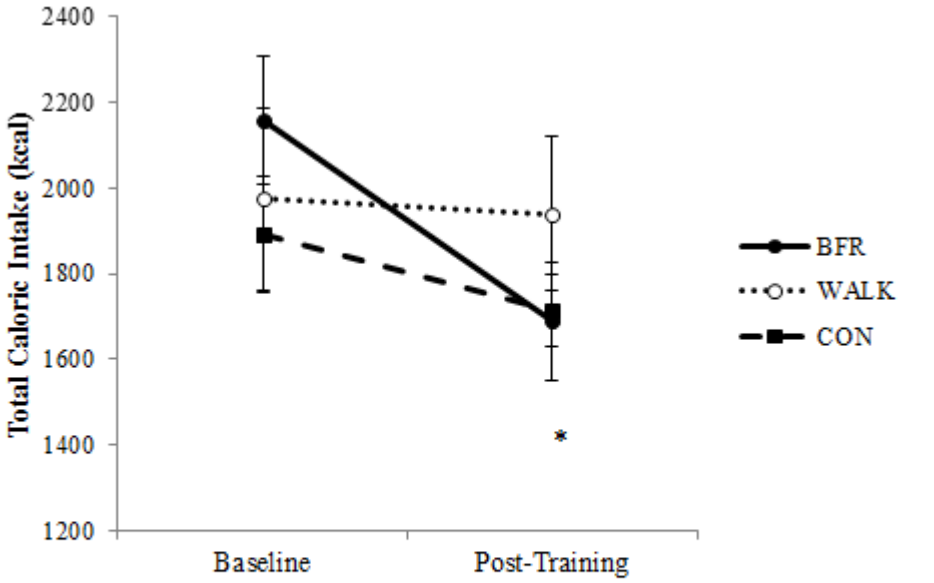


Figure 7. Change in Daily Total Caloric Intake from Week 1 to 12. Values are mean ± SE. Daily caloric intake estmated using 3-day dietary logs administered during week one and week 12. * $p<0.01$, Significant time effect.

DISCUSSION

The main objective of the current study was to assess the effects of 12 weeks walking with blood flow restriction on serum markers of bone turnover (formation/resorption) in college-aged women. Additionally, sub-objectives were to examine changes in muscle strength, calf and thigh MCSA, aerobic capacity, and bone characteristics of the tibia following the 12-week intervention. While the efficacy of BFR exercise to potentiate beneficial neuromuscular adaptations is well known, data on its skeletal effects are lacking. Recently, our group (71) published a report regarding the potential osteogenic effects of low-intensity BFR exercise based on the few existing data in the literature as well as potential mechanisms that might be provided by the blood flow restriction stimulus. However, the conclusions of our report pointed toward a need for further BFR exercise interventions aimed at determining its role in skeletal health and adaptation, especially in female populations. To date, only three studies have examined the chronic bone turnover response to BFR exercise, two utilizing low-intensity resistance training with BFR (53, 55) and one with BFR treadmill walking (10). Moreover, none of these investigations used a female population, which is of significant interest when discussing skeletal health. In their position stand on physical activity and bone health, the American College of Sports Medicine recommends activities such as moderate to high-intensity resistance training as well as weight-bearing activities of high-impact (e.g. jumping, hopping etc.) to maintain or augment bone accrual(57). In this regard, an alternative mode of exercise such as walking with BFR could potentially provide an attractive option for populations not able to perform the high-impact exercise that is currently recommended for bone health. To the best of

the author's knowledge, this is the first intervention in college-aged women designed to evaluate the bone metabolism response to walking with and without blood flow restriction.

Serum Markers of Bone Turnover

Structural bone changes from exercise occur at a relatively slow rate compared to other tissues such as skeletal muscle. For this reason, interventions designed to study structural bone changes require durations > 6 months. However, serum markers of bone turnover respond much sooner to stimuli and help to explain changes in overall bone metabolism. In order for these markers to be clinically useful, it is important to understand what factors can affect their pre-analytical variability including the menstrual cycle, feeding status, caloric energy deficits, time of year (seasonal), age, gender, and acute physical activity (107) . Therefore, it is imperative that as many of these factors are controlled for when evaluating changes over an intervention period. In the current study, only women taking combined oral contraceptives were included, specifically to help control for the fluctuations in bone turnover markers that have been reported to occur during the normal menstrual cycle (126). To help minimize the acute effects of feeding, physical activity, and diurnal fluctuations, blood samples were collected after an overnight fast between the hours of 8:00 and 10:00AM on both collection days. Additionally, the post-training blood sample was collected at least 48 hours after the last exercise session.

In the current study, the bone turnover markers Bone ALP (formation marker) and TRAP5b (resorption marker) were measured at baseline and post-training to determine the effects of walking with BFR on bone turnover. Additionally, Bone ALP

to TRAP5b ratio was calculated as an index of overall bone metabolism. Significant changes in resting serum concentrations of Bone ALP were observed after the 12-week intervention (Table 2). Specifically, post hoc analyses found that both the BFR and CON groups experienced a reduction in serum Bone ALP concentrations following the intervention with no change in the WALK group. With regards to percent change from baseline, only the CON group was significantly different from WALK or BFR (-9.8%, 1.9%, -5.8%) groups (Figure 1). No significant changes were observed for serum TRAP5b or the ratio of Bone ALP to TRAP5b. The results of this study are not in agreement with two of the three published studies on changes in markers of bone turnover following chronic BFR exercise. After 3 weeks (6 days/week) of twice-daily walking with and without BFR, Beekley and colleagues (10) reported a significant increase (10.8%) in resting serum Bone ALP concentrations. This change was only observed in the BFR group. The lack of agreement in results between the study by Beekley and colleagues and the current study may be related to differences in methodologies. Beekley and colleagues (10) had their subjects walk two times per day at a speed of 50 m/min (< 3 mph) for five, two-minute bouts with one-minute rest periods between bouts. The subjects in the current study walked continuously for up to 20 minutes at a speed (3.0-3.8 mph) associated with an intensity of 45% VO_{2peak}. Even though the subjects in the current study were walking at a higher intensity, it is possible that the inserted rest periods between bouts may account for the different Bone ALP response. It is well established that rest periods inserted between mechanical loading cycles amplify the bone loading response and that longer duration loading desensitizes bone cells, acutely (94, 106). Also of importance is the difference in BFR pressures

employed. The study by Beekley et al. (10) started and ended with a higher pressure stimulus (160-230 mmHg) than was used in the current study (140-180 mmHg). Because restriction pressure and the degree of arterial occlusion is highly related to thigh circumference and underlying tissue composition (70), it is likely that the lower occlusion pressures used in our female participants, who likely have smaller thigh circumferences, produced a similar stimulus to the pressures used in the male subjects of Beekley and colleagues (10).

Similar to the results reported by Beekley and colleagues (10), Karabulut and colleagues (53) reported significant increases in resting serum concentrations of Bone ALP following six weeks of low-intensity (20% 1RM) resistance training with blood flow restriction in older men. Also reported was a significant increase in the ratio of Bone ALP to CTX, indicative of a shift in bone metabolism favoring bone formation. Similar to the results of our study, no change in bone resorption markers were observed. It is difficult to directly compare the results of the current study to those of Karabulut and colleagues (53), especially since the modes of exercise are different (resistance training vs. walking) as well as the inherent biological differences in bone turnover markers that arise with age (old vs. young).

While both the studies by Beekley and colleagues (10) and Karabulut and colleagues (53) found significant increases in bone formation markers following BFR exercise, the results of the current study may have been influenced by other factors outside of the exercise protocol. While the utmost care was taken in the design of the current study to minimize outside factors that could potentially affect the outcomes of bone turnover markers, it is possible that the reduction in Bone ALP concentrations

observed in this study were influenced by seasonal and dietary effects. It is well documented that higher concentrations of serum markers of bone formation are generally observed during the winter months than summer months, which is thought to be associated with changes in serum vitamin D during these times (40, 119). The baseline blood samples in this study were obtained in mid-late February and post-training samples were obtained in mid-late May. The significant decrease in Bone ALP concentration observed in the non-exercise control group could be related to this seasonal effect. Furthermore, evidence from the 3-day food logs suggest that caloric energy balance was not maintained over the duration of the study, with the BFR group consuming approximately 400 calories/day less in week 12 compared to week 1 (Table 9). This finding is further supported by a loss in body weight in the BFR group following the 12-week intervention, albeit statistically non-significant (Figure 2). It is well established that short-term caloric restriction and weight loss significantly alters bone formation markers. Ihle and Loucks (50) conducted a study on energy availability and bone turnover in exercising young women and found that bone formation markers were significantly decreased by 10% following 8 days of caloric restriction that resulted in 2 kg loss of body weight. In this study, it could be argued that the treadmill walking may have provided a beneficial bone turnover effect since the WALK group did not experience a weight reduction or a seasonal reduction in Bone ALP concentrations. Furthermore, the potential beneficial effect of treadmill walking on negating the seasonal reduction in Bone ALP in the BFR group could be masked by the effects of the caloric energy deficit and weight loss. However, we do understand that there are limitations to the dietary data collected in this study. The 3-day food logs used to

estimate daily nutrient intakes were only administered twice, once during week one and gain in week 12 of the intervention. Furthermore, we can only assume that participants were accurate and complete in filling out their food logs, which unfortunately, failed to take into account outside supplementation.

Muscle Strength

Several studies have investigated the effects of BFR walking on muscular strength adaptations. However, the current study is the first to report changes in neuromuscular strength following BFR walking in young women. Following 12 weeks of training, isometric knee extension strength at joint angles of 30 and 60 degrees significantly increased over time (Table 4), with no differences between groups. However, the BFR group significantly increased 1RM knee extension strength post-training, with no differences in CON or WALK groups. Similarly, the BFR group had a significantly greater percent change (4.5%) in 1RM knee extension strength compared to CON (-1.9%) post-training (Figure 6). No significant changes post-training were observed for isometric or 1RM knee flexion strength. Similar changes in muscle strength have been reported in other investigations of BFR walking utilizing different study populations.

Ozaki and colleagues (82, 83) conducted two recent studies evaluating the effects of 10 weeks walking with and without BFR. Both studies, one in male and female older adults (n=23) and one in older women only (n=18), used identical exercise protocols (20 minutes walking at 45% heart rate reserve, four days/week for 10 weeks, BFR pressure:140-200 mmHg) which were very similar to the protocol used in our study. In the study using only older women, a significant time effect was reported for

isometric knee extension strength, whereas only the BFR group had a significant increase in dynamic knee extension (8%) and flexion (22%) muscle strength compared to the walking only control group. Our results are strikingly similar to these, with the only exception being that we did not observe a significant increase in dynamic knee flexion. In the other study by Ozaki et al. (82) in older men and women, only dynamic muscle strength of the knee flexors significantly increased (15%) in the BFR group with no difference in dynamic knee extension strength. It should be noted that dynamic knee flexion in our study increased from baseline by almost 5% in the BFR group, whereas the WALK and CON groups experienced a 0.5% and -0.4% percent change from baseline, respectively. However, these group differences were not statistically significant ($p=0.08$). Taken together, the results of the current study and those by Ozaki et al. (82, 83) suggest that continuous treadmill walking at low-intensities (45% HRR, 45%VO_{2peak}) combined with BFR results in significant increases in lower body strength independent of age and gender.

Unlike the protocols mentioned above, Abe et al. (1) investigated the effects of non-continuous walking with and without BFR in young men. Their exercise sessions consisted of five two-minute walking bouts at a speed of 50 m/min with one-minute rest intervals between bouts. Two exercise sessions were completed in one day (morning and afternoon), six days per week for 3 weeks (36 sessions total). The BFR group started at an exercise pressure of 160 mmHg on day one and increased by 10 mmHg each day until a final exercise pressure of 230 mmHg was reached. Following the training intervention, the BFR walk group experienced significant increases in leg press (7.4%) and knee flexion (8.3%) 1RM strength as well isometric knee extension strength

(10.5%) with no post-training strength differences in the CON walk group. Taken together, the results of both our study in young women and that of Abe et al. in young men seem to suggest that both continuous and non-continuous walking with BFR result in positive muscular strength adaptations. However, it is worthwhile to mention that the durations of these studies were very different (12 weeks vs. 3 weeks). Even though the study in young men was only three weeks in duration, the total number of exercise sessions was only slightly less than the total number of sessions in our study (36 sessions vs. 48 sessions, respectively). Overall, the results of our study combined with the other BFR walking studies provide evidence that beneficial adaptations in muscular strength can be achieved from low-intensity, functional exercise such as walking when combined with BFR independent of age and gender.

Body Composition

Unlike the current study, significant increases in mid-thigh MCSA were reported in both of the aforementioned BFR walking studies conducted by Ozaki et al. (82, 83). In their study in older women, the BFR walk group significantly increased MRI-derived mid-thigh MCSA by 3.1% with no change in the CON walk group. Similar improvements were seen in their study in older men and women, where the BFR walk group significantly increased MRI-derived mid-thigh MCSA by 3.2% following the training intervention. In the previously discussed study by Abe and colleagues (1) in young men, MRI-derived mid-thigh MCSA of the quadriceps and hamstrings significantly increased by 5.7% and 7.6%, respectively, in the BFR group with no change in the CON walk group. In an additional study by Abe et al. (2) in older men and women (n=19), estimated muscle-bone CSA of the mid-thigh and proximal

calf (70% of the tibia bone length) were increased by 5.8% and 5.1%, respectively, following 6 weeks of 5 days/week (30 total sessions) BFR walking at 67 m/min (~45%HRR). Each session was 20 minutes in duration employing the same progressive BFR pressures reported in the studies by Ozaki et al. (82, 83).

While each of the previously mentioned studies found significant improvements in mid-thigh MCSA after BFR walking, our study did not. This is somewhat surprising, mainly because our exercise protocol is similar to those used in the majority of the other studies. With the exception of the study by Abe et al. (1) in young men that employed a non-continuous walking protocol of twice daily exercise sessions consisting of five two-minute walking bouts at a lower intensity (50 m/min), our study and the others utilized continuous walking at a similar intensity (45%VO_{2peak} vs. 45% HRR), exercise session duration (20 min), and training frequency (4-5 days/week). One of the main differences between our study and the others was the BFR exercise pressures employed. Our study used an initial pressure of 140 mmHg, which was increased 20 mmHg every 4 weeks with the final exercise pressure reaching 180 mmHg. The studies by Abe et al. (2) and Ozaki et al. (82, 83) in older adults used an initial exercise pressure of 160 mmHg which was increased every day by 10 mmHg until a final exercise pressure of 200 mmHg was reached and used for the remainder of the study duration. Even higher pressures were used in the study by Abe et al. (1) in younger men, where initial exercise pressure started at 160 mmHg and was increased daily by 10 mmHg until a final pressure of 230 mmHg was reached. It is possible that the more conservative restrictive pressures (140-180 mmHg) used in our study did not provide an adequate stimulus to the working musculature. Prescription of BFR pressures and size of the BFR cuff used

are common limitations in the BFR exercise literature, and has been previously discussed in detail in a recent methodological paper (24). Finally, it should be noted that the BFR group in our study had an estimated daily caloric reduction of approximately 400 kcal in week 12 compared to week one. It is well documented that lean muscle mass accretion is influenced by energy availability (97), which could partially explain a lack of mid-thigh MCSA response in the BFR group. Interestingly, we observed increases in MCSA at the tibia 66% site in both BFR and WALK groups. Only the study by Abe et al. (2) in older adults measured MCSA at a lower-leg site (70% of the tibial length) following BFR walking. Estimated bone-muscle CSA significantly increased post-training by 5.1% in the BFR group. However, their study did not employ a CON walking group as did ours. The discrepancy between observed muscle hypertrophy in the lower leg musculature but not the upper leg may be related to the fact that we increased treadmill incline (2% grade) over the walking intervention as a form of progression. Inclined treadmill walking has been shown to increase EMG muscle activity of the plantar flexors as well as power of the ankle joint compared to level walking (63). Although we did not measure muscle activation in our study, it is possible that the increased grade of the treadmill resulted in greater activation and overload of the plantar flexors which contributed to the observed increases in calf MCSA in both the BFR and WALK groups following the training intervention.

Aerobic Capacity

Measures of aerobic capacity following BFR walking have been previously assessed in only a few studies. To the best of our knowledge, this is the first study in young women to report changes in VO_{2peak} following BFR walking exercise. Following

the 12-week intervention, VO_{2peak} remained unchanged in all groups. This finding is in agreement with the previously discussed study by Abe et al. (2) in older adults, which also failed to observe improvements in estimated peak oxygen uptake following six weeks of continuous BFR walking at an intensity of 45% HRR. In contrast, the previously discussed study by Ozaki et al. (83) in older women reported a significant increase in estimated peak oxygen uptake following 10 weeks of walking with and without BFR. Although our study and the other two were conducted at similar exercise intensities, baseline measures of aerobic capacity were lower in the subjects of the study by Ozaki et al. (83), suggesting that baseline cardiovascular fitness was worse compared to our subjects and those of Abe et al. (2). Therefore, it is likely that an intensity of 45% HRR was a large enough stimulus to evoke significant improvements in estimated peak oxygen uptake in this population.

Tibia Bone Characteristics

To the best of the author's knowledge, this is the first study to present changes in bone characteristics of the tibia following BFR exercise. Although changes in the cortical measurements of the tibia after only 12 weeks is unlikely, we hypothesized that changes in the 4% trabecular site may be possible due to the higher metabolic activity associated with this type of bone tissue.

The current study did not find any significant group differences in any of the tibia bone variables following the intervention. However, several time effects were observed at each tibia site. At the tibia 4% site, trabecular bone content and trabecular vBMD both decreased over the study duration (Table 7). Recently, two studies have reported longitudinal data on young women characterizing trabecular bone changes in

the distal tibia. Both Lauretani et al. (62) and Riggs et al. (92) observed significant yearly decreases in trabecular vBMD in women between 20-29 years over a three and six-year period. Our results are in line with these findings, as total bone mineral content and trabecular vBMD both significantly decreased over the intervention period. In contrast, a recent study by Lester et al. (68) reported significant increases in trabecular vBMD at the tibia 4% site following eight weeks of exercise training. In their study, subjects were assigned to one of four groups: high-intensity resistance training, moderate-high-intensity aerobic training, resistance and aerobic training, or non-exercise control. After training 3 days/week for 8 weeks, the aerobic training group increased trabecular vBMD by 1.3% with no changes in the other groups. Interestingly, these results did not resemble the changes they observed in bone turnover markers, as only the resistance training and combined training groups had a significant increase in bone formation markers. These authors also noted that the results should be interpreted with caution, as their observed trabecular vBMD change was within their measurement error. At the very least, it seems the exercise mode and intensity employed in our study was not sufficient to counter-act the age-related loss in trabecular vBMD shown in the previously mentioned longitudinal studies. Our results must also be interpreted with caution, as the length of our intervention was likely too short to reach a full bone remodeling cycle (57).

At the cortical rich 38% site, total bone content and SSI increased following the 12-week intervention (Table 9). At the 66% site, we observed increases in total bone content and area as well as periosteal and endosteal circumference (Table 10). In contrast, total vBMD at the 66% site decreased over time. Taken together, these

changes are indicative of what has previously been reported in longitudinal studies characterizing changes in the diaphyseal sites of the tibia in young women. Recently, a six-year longitudinal study by Lauretani et al. (62) reported that significant periosteal and medullary expansion occurred in women between 20-29 years of age. Our results are consistent with their findings, as we observed an increase in total bone area and periosteal and endosteal circumference. Furthermore, these bone changes are in line with the changes we observed in calf MCSA in both exercise groups. The relationship between muscle and bone has been known for some time, as muscle contractions are considered one of the major bone loading stimuli (34). Therefore, it is reasonable to suggest that the changes in calf MCSA combined with the significant time effects for increases in MVC strength, may in part explain the changes in cortical expansion observed at the 66% site. As with the findings from the 4% site, these bone changes should be interpreted with caution for several reasons. The changes we observed are relatively small (Table 10), and most fall within our measurement precision for detecting a real change. Furthermore, 12 weeks is a relatively short period of time for investigating changes in whole bone tissue. It should be pointed out that the design of the study was not based on measuring whole bone changes, as this was only a secondary purpose. Future studies examining the effects of BFR exercise on organ-level changes should be at least doubled in duration.

CHAPTER V

CONCLUSIONS

The purpose of this study was to investigate the effects of 12 weeks of walking with and without blood flow restriction on metabolic markers of bone turnover in college-aged women. A secondary purpose of the study was to assess changes in thigh and calf MCSA, muscular strength, aerobic capacity, and cortical and trabecular bone characteristics of the tibia following the 12-week intervention. The following research questions were addressed: 1) Will 12 weeks of treadmill walking with and without blood flow restriction significantly alter the resting serum levels of the bone turnover markers Bone ALP and TRAP5b? 2) Will 12 weeks of walking with and without blood flow restriction result in increased thigh and calf MCSA and leg bone free lean body mass? 3) Will 12 weeks of walking with and without blood flow restriction result in increased lower body strength? 4) Will 12 weeks of walking with and without blood flow restriction result in increased aerobic capacity? 5) Will 12 weeks of walking with and without blood flow restriction result in significant changes in bone characteristics of the tibia?

Research Hypothesis 1. 12 weeks of treadmill walking with blood flow restriction but not without will result in significantly elevated levels of Bone ALP. Resting levels of TRAP5b will not change following 12 weeks of treadmill walking with or without blood flow restriction.

No, the results do not support the hypothesis for changes in Bone ALP. Resting serum concentrations of Bone ALP significantly decreased in the BFR group and did

not change in the WALK only group. However, TRAP5b did not change in either group as hypothesized.

Subhypothesis 1. 12 weeks of walking with blood flow restriction will result in increased calf and thigh MCSA and Leg LM, with no changes from walking without blood flow restriction.

No, the results of the study do not support this hypothesis. The BFR and WALK groups both significantly increased calf MCSA. No changes in thigh MCSA occurred in either the BFR or WALK only groups.

Subhypothesis 2. 12 weeks of walking with blood flow restriction will result in increased lower body strength, with no changes from walking without blood flow restriction.

Yes, the results of the current study support this hypothesis**.** The BFR group significantly increased knee extension 1RM strength. Additionally, significant time effects occurred for MVC knee extensions at joint angles of 30 and 60 degrees. No changes in knee flexion 1RM or MVC strength occurred in any group.

Subhypothesis 3. 12 weeks of walking with blood flow restriction will result in increased aerobic capacity but will remain unchanged following 12 weeks of walking without blood flow restriction.

No, aerobic capacity did not change in either group following the intervention.

Subhypothesis 4. 12 weeks of walking with blood flow restriction will result in positive bone adaptations at the distal tibia site with no change after 12 weeks of walking without blood flow restriction.

This hypothesis was not supported. No beneficial adaptations occurred at the 4% tibia site in the BFR group following the intervention.

Significance of the Study

The results of the current study suggest that walking with BFR does not cause favorable adaptations is resting serum levels of bone turnover markers in young women taking combined oral contraceptives. However, there were some potential unforeseen factors that might have affected the levels of bone turnover markers including caloric restriction/weight loss over the 12-week intervention as well as potential seasonal effects from starting the protocol in the winter and ending toward the beginning of summer. Walking with BFR did result in beneficial neuromuscular adaptations including increased muscle strength of the knee extensors and increased MCSA of the plantar flexor muscles. Populations undergoing rehabilitation or severely deconditioned individuals could benefit from this functional form of exercise with regards to neuromuscular adaptations. However, there is not enough evidence from the current data to recommend its use for skeletal health.

Future Research

Research into the potential skeletal benefits of BFR exercise should not cease because of the results of this study. There is good evidence from research in young men and older adults that there is potential for positive bone adaptations to occur from exercise with BFR. Future research may want to examine resistance training as the primary mode of exercise used in conjunction with BFR to test its effects on bone metabolism and organ-level bone changes in women across the age span. In addition, proper exercise prescription following the known rules for bone adaptation to loading

(short bouts with rest periods) should be considered when designing BFR studies aimed at improving the bone response. Lastly, great care must be taken to control for known factors that contribute to pre-analytical variability in bone turnover markers to ensure that the changes are related to the intervention. In addition, nutrient intake should be more tightly regulated so that weight loss does not occur over the intervention. The current study only used a 3-day food log at two time points over the twelve week intervention. Future studies could assess nutrient intake more often as well as assess weight change at several intervals throughout the intervention. Lastly, BFR pressures need to be considered. It is likely that the prescribed pressures need to be individualized to the person rather than the population. However, further research in this area is needed.

REFERENCES

1. Abe T, Kearns CF, and Sato Y. Muscle size and strength are increased following walk training with restricted venous blood flow from the leg muscle, Kaatsu-walk training. *J. Appl. Physiol.* 2006;100(5):1460.

2. Abe T, Sakamaki M, Fujita S, Ozaki H, Sugaya M, Sato Y, and Nakajima T. Effects of Low-Intensity Walk Training With Restricted Leg Blood Flow on Muscle Strength and Aerobic Capacity in Older Adults. *J. Geriatr. Phys. Ther.* 2010;33(1):34.

3. Abe T, Yasuda T, Midorikawa T, Sato Y, Kearns C, Inoue K, Koizumi K, and Ishii N. Skeletal muscle size and circulating IGF-1 are increased after two weeks of twice daily "KAATSU" resistance training. *Int. J. KAATSU Training Res.* 2005;1(1):6-12.

4. Adami S, Gatti D, Viapiana O, Fiore CE, Nuti R, Luisetto G, Ponte M, and Rossini M. Physical activity and bone turnover markers: a cross-sectional and a longitudinal study. *Calcif. Tissue Int.* 2008;83(6):388-92.

5. Ammann P, and Rizzoli R. Bone strength and its determinants. *Osteoporosis Int.* 2003;14:13-8.

6. Baim S, Binkley N, Bilezikian JP, Kendler DL, Hans DB, Lewiecki EM, and Silverman S. Official positions of the International Society for Clinical Densitometry and executive summary of the 2007 ISCD Position Development Conference. *J. Clin. Densitometry*. 2008;11(1):75-91.

7. Bassey E, Rothwell M, Littlewood J, and Pye D. Pre and Postmenopausal Women Have Different Bone Mineral Density Responses to the Same High Impact Exercise. *J. Bone Miner. Res.* 1998;13(12):1805-13.

8. Bauer D, Garnero P, Bilezikian J, Greenspan S, Ensrud K, Rosen C, Palermo L, and Black D. Short-term changes in bone turnover markers and bone mineral density response to parathyroid hormone in postmenopausal women with osteoporosis. *J. Clin. Endocrinol. Metab.* 2006;91(4):1370.

9. Beck BR, Kent K, Holloway L, and Marcus R. Novel, high-frequency, low-strain mechanical loading for premenopausal women with low bone mass: early findings. *J. Bone Miner. Metab.* 2006;24(6):505-7.

10. Beekley M, Sato Y, and Abe T. KAATSU-walk training increases serum bone-specific alkaline phosphatase in young men. *Int. J. KAATSU Training Res.* 2005;1(2):77-81.

11. Bemben D, Palmer I, Abe T, Sato Y, and Bemben M. Effects of a single bout of low intensity KAATSU resistance training on markers of bone turnover in young men. *Int. J. KAATSU Training Res.* 2007;3(2):21-6.

12. Bonewald LF, and Johnson ML. Osteocytes, mechanosensing and Wnt signaling. *Bone*. 2008;42(4):606-15.

13. Brown EM. Calcium receptor and regulation of parathyroid hormone secretion. *Rev Endocr Metab Disord*. 2000;1(4):307-15.

14. Carter PH, and Schipani E. The roles of parathyroid hormone and calcitonin in bone remodeling: prospects for novel therapeutics. *Endocrine, Metabolic &# 38; Immune Disorders-Drug Targets (Formerly Current Dru*. 2006;6(1):59-76.

15. Cavanagh PR, and Lafortune MA. Ground reaction forces in distance running. *J. Biomech.* 1980;13(5):397-406.

16. Clarke B. Normal bone anatomy and physiology. *Clin. J. Am. Soc. Nephrol.* 2008;3(Supplement 3):S131.

17. Courteix D, Lespessailles E, Peres SL, Obert P, Germain P, and Benhamou C. Effect of physical training on bone mineral density in prepubertal girls: a comparative study between impact-loading and non-impact-loading sports. *Osteoporosis Int.* 1998;8(2):152-8.

18. Datta HK, Ng WF, Walker JA, Tuck SP, and Varanasi SS. The cell biology of bone metabolism. *J. Clin. Pathol.* 2008;61(5):577-87.

19. Davee AM, Rosen CJ, and Adler RA. Exercise patterns and trabecular bone density in college women. *J. Bone Miner. Res.* 1990;5(3):245-50.

20. Delmas P, Eastell R, Garnero P, Seibel M, and Stepan J. The use of biochemical markers of bone turnover in osteoporosis. *Osteoporosis Int.* 2000;11:2-17.

21. Devlin MJ, and Lieberman DE. Variation in estradiol level affects cortical bone growth in response to mechanical loading in sheep. *J. Exp. Biol.* 2007;210:602-13.

22. Downey PA, and Siegel MI. Bone biology and the clinical implications for osteoporosis. *Phys. Ther.* 2006;86(1):77.

23. Dufek JS, and Bates BT. The evaluation and prediction of impact forces during landings. *Med. Sci. Sports Exerc.* 1990;22(3):370.

24. Fahs CA, Loenneke JP, Rossow LM, Thiebaud RS, and Bemben MG. Methodological considerations for blood flow restricted resistance exercise. *Journal of Trainology*. 2012;1:14-22.

25. Fehling P, Alekel L, Clasey J, Rector A, and Stillman R. A comparison of bone mineral densities among female athletes in impact loading and active loading sports. *Bone*. 1995;17(3):205-10.

26. Felsenberg D, and Boonen S. The bone quality framework: Determinants of bone strength and their interrelationships, and implications for osteoporosis management*. *Clin. Ther.* 2005;27(1):1-11.

27. Frank A, Lorbergs A, Chilibeck P, Farthing J, and Kontulainen S. Muscle cross sectional area and grip torque contraction types are similarly related to pQCT derived bone strength indices in the radii of older healthy adults. *J Musculoskelet Neuronal Interact*. 2010;10(2):136-41.

28. Friedlander AL, Genant HK, Sadowsky S, Byl NN, and Glüer CC. A two year program of aerobics and weight training enhances bone mineral density of young women. *J. Bone Miner. Res.* 1995;10(4):574-85.

29. Fritton S, and Rubin C. In vivo measurement of bone deformations using strain gages. *Bone mechanics handbook, 2nd edition. Boca Raton: CRC Press. p*. 2001:8–91.

30. Fritton SP. Quantifying the strain history of bone: spatial uniformity and self-similarity of low-magnitude strains. *J. Biomech.* 2000;33(3):317-25.

31. Fritton SP, and Weinbaum S. Fluid and Solute Transport in Bone: Flow-Induced Mechanotransduction. *Annu Rev Fluid Mech*. 2009;41:347-74.

32. Frost HM. Bone "mass" and the "mechanostat": a proposal. *Anat. Rec.* 1987;219(1):1-9.

33. Frost HM. The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. *Bone Miner.* 1987;2(2):73-85.

34. Frost HM. A 2003 update of bone physiology and Wolff's Law for clinicians. *Angle Orthod*. 2004;74(1):3-15.

35. Fujita T, Brechue W, Kurita K, Sato Y, and Abe T. Increased muscle volume and strength following six days of low-intensity resistance training with restricted muscle blood flow. *Int. J. KAATSU Training Res.* 2008;4(1):1-8.

36. Garnero P, Sornay Rendu E, Chapuy MC, and Delmas PD. Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. *J. Bone Miner. Res.* 1996;11(3):337-49.

37. Gass M, and Dawson-Hughes B. Preventing osteoporosis-related fractures: an overview. *The American journal of medicine*. 2006;119(4):S3-S11.

38. General S. Bone health and osteoporosis: a report of the Surgeon General. *US Department of Health and Human Services, Rockville, MD, USA*. 2004.

39. Gleeson PB, Protas EJ, Leblanc AD, Schneider VS, and Evans HJ. Effects of weight lifting on bone mineral density in premenopausal women. *J. Bone Miner. Res.* 1990;5(2):153-8.

40. Glover S, Garnero P, Naylor K, Rogers A, and Eastell R. Establishing a reference range for bone turnover markers in young, healthy women. *Bone*. 2008;42(4):623-30.

41. Gracia CR, Sammel MD, Freeman EW, Lin H, Langan E, Kapoor S, and Nelson DB. Defining menopause status: creation of a new definition to identify the early changes of the menopausal transition. *Menopause*. 2005;12(2):128.

42. Greenspan SL, Resnick NM, and Parker RA. Early changes in biochemical markers of bone turnover are associated with long-term changes in bone mineral density in elderly women on alendronate, hormone replacement therapy, or combination therapy: a three-year, double-blind, placebo-controlled, randomized clinical trial. *J. Clin. Endocrinol. Metab.* 2005;90(5):2762.

43. Haapasalo H, Kannus P, Sievanen H, Pasanen M, Uusi-Rasi K, Heinonen A, Oja P, and Vuori I. Effect of long-term unilateral activity on bone mineral density of female junior tennis players. *J. Bone Miner. Res.* 1998;13(2):310-9.

44. Hadjidakis DJ, and Androulakis, II. Bone remodeling. *Ann. N. Y. Acad. Sci.* 2006;1092:385-96.

45. Hawker G. The epidemiology of osteoporosis. *The Journal of rheumatology. Supplement*. 1996;45:2.

46. Heinonen A, Oja P, Kannus P, Sievanen H, Manttari A, and Vuori I. Bone mineral density of female athletes in different sports. *Bone Miner.* 1993;23(1):1-14.

47. Heinonen A, Sievänen H, Kannus P, Oja P, and Vuori I. Site-specific skeletal response to long-term weight training seems to be attributable to principal

loading modality: a pQCT study of female weightlifters. *Calcif. Tissue Int.* 2002;70(6):469-74.

48. Heinrich CH, Going SB, Pamenter RW, Perry CD, Boyden TW, and Lohman TG. Bone mineral content of cyclically menstruating female resistance and endurance trained athletes. *Med. Sci. Sports Exerc.* 1990;22(5):558.

49. Hughes JM, and Petit MA. Biological underpinnings of Frost's mechanostat thresholds: the important role of osteocytes. *J Musculoskelet Neuronal Interact*. 2010;10(2):128-35.

50. Ihle R, and Loucks AB. Dose-response relationships between energy availability and bone turnover in young exercising women. *J. Bone Miner. Res.* 2004;19(8):1231-40.

51. Iida H, Kurano M, Takano H, Kubota N, Morita T, Meguro K, Sato Y, Abe T, Yamazaki Y, and Uno K. Hemodynamic and neurohumoral responses to the restriction of femoral blood flow by KAATSU in healthy subjects. *Eur. J. Appl. Physiol.* 2007;100(3):275-85.

52. Kanis JA, and Kanis J. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. *Osteoporosis Int.* 1994;4(6):368-81.

53. Karabulut M, Bemben DA, Sherk VD, Anderson MA, Abe T, and Bemben MG. Effects of high-intensity resistance training and low-intensity resistance training with vascular restriction on bone markers in older men. *Eur. J. Appl. Physiol.* 2011:1-9.

54. Kato Y, Windle JJ, Koop BA, Mundy GR, and Bonewald LF. Establishment of an osteocyte-like cell line, MLO-Y4. *J. Bone Miner. Res.* 1997;12(12):2014-23.

55. Kim SJ, Sherk VD, Bemben MG, and Bemben DA. Effects of Short Term Low Intensity Resistance Training with Blood Flow Restriction on Bone Markers and Muscle Cross-Sectional Area in Young Men. *International Journal of Exercise Science*. 2012;5(2):6.

56. Klibanski A, Adams-Campbell L, Bassford T, Blair S, Boden S, Dickersin K, Gifford D, Glasse L, Goldring S, and Hruska K. Osteoporosis prevention, diagnosis, and therapy. *JAMA*. 2001;285(6):785-95.

57. Kohrt WM, Bloomfield SA, Little KD, Nelson ME, and Yingling VR. Physical activity and bone health. *Med. Sci. Sports Exerc.* 2004;36(11):1985.

58. Kramer I, Halleux C, Keller H, Pegurri M, Gooi JH, Weber PB, Feng JQ, Bonewald LF, and Kneissel M. Osteocyte Wnt/beta-catenin signaling is required for normal bone homeostasis. *Mol. Cell. Biol.* 2010;30(12):3071-85.

59. Krolner B, and Toft B. Vertebral bone loss: an unheeded side effect of therapeutic bed rest. *Clin Sci (Lond)*. 1983;64(5):537-40.

60. Lane NE. Epidemiology, etiology, and diagnosis of osteoporosis. *Am. J. Obstet. Gynecol.* 2006;194(2):S3-S11.

61. Lanyon L, Armstrong V, Saxon L, Sunters A, Sugiyama T, Zaman G, and Price J. Estrogen receptors critically regulate bones' adaptive responses to loading. *Clin. Rev. Bone. Miner. Metab.* 2007;5(4):234-48.

62. Lauretani F, Bandinelli S, Griswold ME, Maggio M, Semba R, Guralnik JM, and Ferrucci L. Longitudinal Changes in BMD and Bone Geometry in a Population-Based Study. *J. Bone Miner. Res.* 2008;23(3):400-8.

63. Lay AN, Hass CJ, Richard Nichols T, and Gregor RJ. The effects of sloped surfaces on locomotion: an electromyographic analysis. *J. Biomech.* 2007;40(6):1276-85.

64. LeBlanc A, Schneider V, Shackelford L, West S, Oganov V, Bakulin A, and Voronin L. Bone mineral and lean tissue loss after long duration space flight. *J Musculoskelet Neuronal Interact*. 2000;1(2):157-60.

65. Lee K, Jessop H, Suswillo R, Zaman G, and Lanyon L. Endocrinology: bone adaptation requires oestrogen receptor. *Nature*. 2003;424(6947):389-.

66. Leibbrandt A, and Penninger JM. RANK/RANKL: regulators of immune responses and bone physiology. *Ann. N. Y. Acad. Sci.* 2008;1143:123-50.

67. Leibson CL, Tosteson ANA, Gabriel SE, Ransom JE, and Melton III LJ. Mortality, Disability, and Nursing Home Use for Persons with and without Hip Fracture: A Population Based Study. *J. Am. Geriatr. Soc.* 2002;50(10):1644-50.

68. Lester ME, Urso ML, Evans RK, Pierce JR, Spiering BA, Maresh CM, Hatfield DL, Kraemer WJ, and Nindl BC. Influence of exercise mode and osteogenic index on bone biomarker responses during short-term physical training. *Bone*. 2009;45(4):768-76.

69. Loenneke J, Fahs C, Rossow L, Abe T, and Bemben M. The anabolic benefits of venous blood flow restriction training may be induced by muscle cell swelling. *Med. Hypotheses*. 2011.

70. Loenneke JP, Fahs CA, Rossow LM, Sherk VD, Thiebaud RS, Abe T, Bemben DA, and Bemben MG. Effects of cuff width on arterial occlusion: implications for blood flow restricted exercise. *Eur. J. Appl. Physiol.* 2011:1-10.

71. Loenneke JP, Young KC, Fahs CA, Rossow LM, Bemben DA, and Bemben MG. Blood flow restriction: Rationale for improving bone. *Med. Hypotheses*. 2012.

72. Lohman T, Going S, Pamenter R, Hall M, Boyden T, Houtkooper L, Ritenbaugh C, Bare L, Hill A, and Aickin M. Effects of resistance training on regional and total bone mineral density in premenopausal women: a randomized prospective study. *J. Bone Miner. Res.* 1995;10(7):1015-24.

73. Macdonald H, Kontulainen S, Petit M, Janssen P, and McKay H. Bone strength and its determinants in pre-and early pubertal boys and girls. *Bone*. 2006;39(3):598-608.

74. MacLean C, Newberry S, Maglione M, McMahon M, Ranganath V, Suttorp M, Mojica W, Timmer M, Alexander A, and McNamara M. Systematic review: comparative effectiveness of treatments to prevent fractures in men and women with low bone density or osteoporosis. *Ann. Intern. Med.* 2008;148(3):197.

75. MacNeil JA, and Boyd SK. Accuracy of high-resolution peripheral quantitative computed tomography for measurement of bone quality. *Med. Eng. Phys.* 2007;29(10):1096-105.

76. Madarame H, Sasaki K, and Ishii N. Endocrine responses to upper-and lower-limb resistance exercises with blood flow restriction. *Acta Physiol. Hung.* 2010;97(2):192-200.

77. Manini TM, and Clark BC. Blood flow restricted exercise and skeletal muscle health. *Exerc. Sport Sci. Rev.* 2009;37(2):78.

78. McGuigan F, Murray L, Gallagher A, Davey Smith G, Neville CE, Van't Hof R, Boreham C, and Ralston SH. Genetic and environmental determinants of peak bone mass in young men and women. *J. Bone Miner. Res.* 2002;17(7):1273-9.

79. Nakajima T, Kurano M, Iida H, Takano H, Oonuma H, Morita T, Meguro K, Sato Y, and Nagata T. Use and safety of KAATSU training: results of a national survey. *Int. J. KAATSU Training Res.* 2006;2(1):5-13.

80. Nakamura T, Imai Y, Matsumoto T, Sato S, Takeuchi K, Igarashi K, Harada Y, Azuma Y, Krust A, and Yamamoto Y. Estrogen Prevents Bone Loss via Estrogen Receptor [alpha] and Induction of Fas Ligand in Osteoclasts. *Cell*. 2007;130(5):811-23.

81. Nappi C, Di Spiezio Sardo A, Acunzo G, Bifulco G, Tommaselli G, Guida M, and Di Carlo C. Effects of a low-dose and ultra-low-dose combined oral contraceptive use on bone turnover and bone mineral density in young fertile women: a prospective controlled randomized study. *Contraception*. 2003;67(5):355-9.

82. Ozaki H, Miyachi M, Nakajima T, and Abe T. Effects of 10 Weeks Walk Training With Leg Blood Flow Reduction on Carotid Arterial Compliance and Muscle Size in the Elderly Adults. *Angiology*. 2011;62(1):81.

83. Ozaki H, Sakamaki M, Yasuda T, Fujita S, Ogasawara R, Sugaya M, Nakajima T, and Abe T. Increases in thigh muscle volume and strength by walk training with leg blood flow reduction in older participants. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*. 2011;66(3):257.

84. Ozcivici E, Luu YK, Adler B, Qin YX, Rubin J, Judex S, and Rubin CT. Mechanical signals as anabolic agents in bone. *Nat. Rev. Rheumatol.* 2010;6(1):50-9.

85. Patterson SD, and Ferguson RA. Increase in calf post-occlusive blood flow and strength following short-term resistance exercise training with blood flow restriction in young women. *Eur. J. Appl. Physiol.* 2010;108(5):1025-33.

86. Pierce JR, Clark BC, Ploutz-Snyder LL, and Kanaley JA. Growth hormone and muscle function responses to skeletal muscle ischemia. *J. Appl. Physiol.* 2006;101(6):1588.

87. Porthouse J, Cockayne S, King C, Saxon L, Steele E, Aspray T, Baverstock M, Birks Y, Dumville J, and Francis R. Randomised controlled trial of calcium and supplementation with cholecalciferol (vitamin D3) for prevention of fractures in primary care. *BMJ*. 2005;330(7498):1003.

88. Post TM, Cremers SC, Kerbusch T, and Danhof M. Bone physiology, disease and treatment: towards disease system analysis in osteoporosis. *Clin. Pharmacokinet.* 2010;49(2):89-118.

89. Price C, Zhou X, Li W, and Wang L. Real-time measurement of solute transport within the lacunar-canalicular system of mechanically loaded bone: direct evidence for load-induced fluid flow. *J. Bone Miner. Res.* 2011;26(2):277-85.

90. Raggatt LJ, and Partridge NC. Cellular and molecular mechanisms of bone remodeling. *J. Biol. Chem.* 2010;285(33):25103-8.

91. Reeves GV, Kraemer RR, Hollander DB, Clavier J, Thomas C, Francois M, and Castracane VD. Comparison of hormone responses following light resistance exercise with partial vascular occlusion and moderately difficult resistance exercise without occlusion. *J. Appl. Physiol.* 2006;101(6):1616.

92. Riggs BL, Melton III LJ, Robb RA, Camp JJ, Atkinson EJ, McDaniel L, Amin S, Rouleau PA, and Khosla S. A Population-Based Assessment of Rates of Bone Loss at Multiple Skeletal Sites: Evidence for Substantial Trabecular Bone Loss in Young Adult Women and Men. *J. Bone Miner. Res.* 2008;23(2):205-14.

93. Robinson T, Snow Harter C, Taaffe D, Gillis D, Shaw J, and Marcus R. Gymnasts exhibit higher bone mass than runners despite similar prevalence of amenorrhea and oligomenorrhea. *J. Bone Miner. Res.* 1995;10(1):26-35.

94. Robling AG, Burr DB, and Turner CH. Recovery periods restore mechanosensitivity to dynamically loaded bone. *J. Exp. Biol.* 2001;204(19):3389-99.

95. Robling AG, Niziolek PJ, Baldridge LA, Condon KW, Allen MR, Alam I, Mantila SM, Gluhak-Heinrich J, Bellido TM, Harris SE, and Turner CH. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. *J. Biol. Chem.* 2008;283(9):5866-75.

96. Ross AC, Manson JAE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, and Jones G. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J. Clin. Endocrinol. Metab.* 2011;96(1):53-8.

97. Ross R, Pedwell H, and Rissanen J. Effects of energy restriction and exercise on skeletal muscle and adipose tissue in women as measured by magnetic resonance imaging. *The American journal of clinical nutrition*. 1995;61(6):1179-85.

98. Rubin C, Turner AS, Müller R, Mittra E, McLeod K, Lin W, and Qin YX. Quantity and quality of trabecular bone in the femur are enhanced by a strongly anabolic, noninvasive mechanical intervention. *J. Bone Miner. Res.* 2002;17(2):349-57.

99. Sapega AA, Heppenstall RB, Chance B, Park Y, and Sokolow D. Optimizing tourniquet application and release times in extremity surgery. A biochemical and ultrastructural study. *J. Bone Joint Surg. Am.* 1985;67(2):303.

100. Sato Y. The history and future of KAATSU training. *Int. J. KAATSU Training Res.* 2005;1(1):1-5.

101. Schönau E. The development of the skeletal system in children and the influence of muscular strength. *Hormone Research in Paediatrics*. 2000;49(1):27-31.

102. Shaw JA, and Murray DG. The relationship between tourniquet pressure and underlying soft-tissue pressure in the thigh. *J. Bone Joint Surg. Am.* 1982;64(8):1148.

103. Shibata Y, Ohsawa I, Watanabe T, Miura T, and Sato Y. Effects of physical training on bone mineral density and bone metabolism. *J Physiol Anthropol Appl Human Sci*. 2003;22(4):203-8.

104. Sinaki M, Wahner H, Bergstralh E, Hodgson S, Offord K, Squires R, Swee R, and Kao P. Three-year controlled, randomized trial of the effect of dose-specified loading and strengthening exercises on bone mineral density of spine and femur in nonathletic, physically active women. *Bone*. 1996;19(3):233-44.

105. Snow Harter C, Bouxsein ML, Lewis BT, Carter DR, and Marcus R. Effects of resistance and endurance exercise on bone mineral status of young women: a randomized exercise intervention trial. *J. Bone Miner. Res.* 1992;7(7):761-9.

106. Srinivasan S, Weimer DA, Agans SC, Bain SD, and Gross TS. Low-Magnitude Mechanical Loading Becomes Osteogenic When Rest Is Inserted Between Each Load Cycle. *J. Bone Miner. Res.* 2002;17(9):1613-20.

107. Szulc P, and Delmas P. Biochemical markers of bone turnover: potential use in the investigation and management of postmenopausal osteoporosis. *Osteoporosis Int.* 2008;19(12):1683-704.

108. Takano H, Morita T, Iida H, Asada K, Kato M, Uno K, Hirose K, Matsumoto A, Takenaka K, and Hirata Y. Hemodynamic and hormonal responses to a short-term low-intensity resistance exercise with the reduction of muscle blood flow. *Eur. J. Appl. Physiol.* 2005;95(1):65-73.

109. Takarada Y, Nakamura Y, Aruga S, Onda T, Miyazaki S, and Ishii N. Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. *J. Appl. Physiol.* 2000;88(1):61.

110. Takarada Y, Takazawa H, Sato Y, Takebayashi S, Tanaka Y, and Ishii N. Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. *J. Appl. Physiol.* 2000;88(6):2097.

111. Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K, Ito M, Takeshita S, and Ikeda K. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab*. 2007;5(6):464-75.

112. Turner CH. Three rules for bone adaptation to mechanical stimuli. *Bone*. 1998;23(5):399-407.

113. Turner CH, Yoshikawa T, Forwood MR, Sun T, and Burr DB. High frequency components of bone strain in dogs measured during various activities. *J. Biomech.* 1995;28(1):39-44.

114. Tzelepi V, Tsamandas AC, Zolota V, and Scopa CD. Bone Anatomy, Physiology and Function. *Bone Metastases*. 2009:3-30.

115. Vainionpää A, Korpelainen R, Leppäluoto J, and Jämsä T. Effects of high-impact exercise on bone mineral density: a randomized controlled trial in premenopausal women. *Osteoporosis Int.* 2005;16(2):191-7.

116. Wang Y, McNamara LM, Schaffler MB, and Weinbaum S. A model for the role of integrins in flow induced mechanotransduction in osteocytes. *Proc. Natl. Acad. Sci. U. S. A.* 2007;104(40):15941-6.

117. Whedon GD. Disuse osteoporosis: physiological aspects. *Calcif. Tissue Int.* 1984;36 Suppl 1:S146-50.

118. Winters-Stone KM, and Snow CM. Site-specific response of bone to exercise in premenopausal women. *Bone*. 2006;39(6):1203-9.

119. Woitge HW, Scheidt-Nave C, Kissling C, Leidig-Bruckner G, Meyer K, Grauer A, Scharla SH, Ziegler R, and Seibel MJ. Seasonal variation of biochemical indexes of bone turnover: results of a population-based study. *J. Clin. Endocrinol. Metab.* 1998;83(1):68-75.

120. Wolff J. The Classic: On the Inner Architecture of Bones and its Importance for Bone Growth. *Clin. Orthop. Relat. Res.* 2010;468(4):1056-65.

121. Wu J, Ishizaki S, Kato Y, Kuroda Y, and Fukashiro S. The side-to-side differences of bone mass at proximal femur in female rhythmic sports gymnasts. *J. Bone Miner. Res.* 1998;13(5):900-6.

122. Yasuda T, Brechue W, Fujita T, Shirakawa J, Sato Y, and Abe T. Muscle activation during low-intensity muscle contractions with restricted blood flow. *J. Sports Sci.* 2009;27(5):479-89.

123. You J, Yellowley C, Donahue H, Zhang Y, Chen Q, and Jacobs C. Substrate deformation levels associated with routine physical activity are less stimulatory to bone cells relative to loading-induced oscillatory fluid flow. *J. Biomech. Eng.* 2000;122:387.

124. Young KC, Sherk VD, and Bemben DA. Inter-limb musculoskeletal differences in competitive ten-pin bowlers: A preliminary analysis. *J Musculoskelet Neuronal Interact*. 2011;11(1):21-6.

125. Zallone A. Direct and indirect estrogen actions on osteoblasts and osteoclasts. *Ann. N. Y. Acad. Sci.* 2006;1068:173-9.

126. Zittermann A, Schwarz I, Scheld K, Sudhop T, Berthold H, Von Bergmann K, Van der Ven H, and Stehle P. Physiologic fluctuations of serum estradiol levels influence biochemical markers of bone resorption in young women. *J. Clin. Endocrinol. Metab.* 2000;85(1):95.

APPENDICES

Appendix A. Recruitment Materials 101

Appendix B. Initial Screening Checklist 105

Appendix C. Informed Consent and Authorization to use or Disclose Protected Health Information 108

Appendix D. Study Questionnaires 119

Appendix E. IRB Approval Letter 131

Appendix F. Exercise Protocol 133

Appendix G. Raw Data 134

Appendix A

Recruitment Materials

Female Participants Needed!

Have you wanted to start a regular exercise program? Would you like to receive information on your body composition and bone density? Come join our study! Monetary compensation is available.

Purpose: To investigate the effects of 12 weeks walk training with and without blood flow reduction on bone health, muscle mass, strength, and aerobic fitness in females aged 18-30 yrs.

Who: Women between the ages of 18 to 30 years who are currently taking oral contraceptives, generally healthy, and have not been exercising regularly (more than twice per week) for the past 3 months.

Visits: pre and post testing visits include bone density and body composition scans, lower body strength, muscle cross-sectional area, aerobic fitness testing, and blood draws.

This study involves low-dose radiation that will allow us to measure total body muscle and fat mass, bone density, and thigh muscle cross-sectional area.

Participants will be randomly assigned into 1 of 3 groups. Exercise groups will train 4 times per week for 12 weeks, 20 min per session.

Where: Bone Density Laboratory, Department of Health and Exercise Science, University of Oklahoma.

If you are interested or have further questions, please contact the individual below:

Kaelin Young

Department of Health and Exercise Science

kcyoung@ou.edu

The University of Oklahoma is an equal opportunity institution.

Name: Kaelin Young
Phone: (253)255-5617
e-mail: kcyoung@ou.edu

Verbal Recruitment Script

Hello, my name is ----------, and I am a graduate student in the Department of Health and Exercise Science at the University of Oklahoma. I am inviting you to participate in a research study we are conducting. The title of the study is "The effects of 12 weeks walking with and without blood flow reduction on bone metabolic markers in young women." We are specifically looking for women between the ages of 18-30 years who are not currently exercising more than 2 days per week and are taking a combined oral contraceptive pill. We are performing this research study to determine if combining blood flow reduction with walking impacts bone health, muscle mass, strength, and aerobic fitness in women ages 18-30 years. Blood flow reduction during exercise is a new training technique using specially designed cuffs (5 cm width) which are placed around the most upper portion of each leg and are inflated to reduce blood flow during exercise.

You will be randomly assigned to one of three groups: low-intensity treadmill walking with blood flow reduction, low-intensity treadmill walking control, or a non-exercise control group.

The total time commitment for this study is 14 weeks. The two exercise groups will train 4 times/week for 12 weeks with each session lasting about 25 minutes. All three groups will be measured at 2 time intervals (baseline and post-training). During pre and post testing, we will measure your bone density, lower-body strength, lean and fat body mass, thigh and calf muscle cross-sectional area, aerobic fitness, and you will have your blood sampled by a trained phlebotomist.

The pre and post testing sessions will involve exposure to low-dose radiation (DXA and peripheral quantitative computerized tomography) to obtain measures of muscle mass, fat, bone density, and thigh and calf muscle cross-sectional area.

There is a possibility of mild soreness because of the testing and exercise, but any discomfort should be gone within a couple of days. Additionally, there may be some discomfort and mild subcutaneous bruising associated with the blood draws which will be performed by a nurse or trained phlebotomist. Monetary compensation for your participation is available.

I would be happy to answer any questions that you may have about the study.

E-mail Message

I am inviting you to participate in a research study we are conducting in the Department of Health and Exercise Science. The title of the study is "The effects of 12 weeks walking with and without blood flow reduction on bone metabolic markers in young women". We are specifically looking for women between the ages of 18-30 years who are not currently exercising more than 2 days per week and are taking a combined oral contraceptive pill. We are performing this research study to determine if combining blood flow reduction with walking impacts bone health, muscle mass, strength, and aerobic fitness in women ages 18-30 years. Blood flow reduction during exercise is a new training technique using specially designed cuffs (5 cm width) which are placed around the most upper portion of each leg and are inflated to reduce blood flow during exercise.

If you decide to participate, you will be randomly assigned to one of three groups: low-intensity treadmill walking with blood flow reduction, low-intensity treadmill walking control, or a non-exercise control group.

The total time commitment for this study is 14 weeks. The two exercise groups will train 4 times/week for 12 weeks with each session lasting about 25 minutes. All three groups will be measured at 2 time intervals (baseline and post training). During pre and post testing sessions, we will measure your bone mineral density, lower body strength, lean and fat body mass and percentage, thigh and calf muscle cross-sectional area, aerobic fitness, and you will have your blood sampled by a trained phlebotomist. The testing sessions will involve exposure to low-dose radiation (DXA and peripheral quantitative computerized tomography) to obtain measures of muscle mass, fat, bone density, and thigh muscle cross-sectional area.

There is a possibility of mild soreness because of the testing and exercise, but any discomfort should be gone within a couple of days. Additionally, there may be some discomfort or mild subcutaneous bruising at the site of the blood draws which will be performed by a nurse or trained phlebotomist.

Thank you for your consideration to participate in our study. Monetary compensation is available for participation in this study. If interested or have further questions, please contact **Kaelin Young (kcyoung@ou.edu).**

"The OU IRB has approved the content of this message but not the method of distribution. The OU IRB has no authority to approve distribution by mass email."

Warm Regards,

Appendix B

Initial Screening Checklist

Subject Screening Recruitment Form

University of Oklahoma Bone Density Laboratory

"Effects of 12 Weeks Walking with or without Blood Flow Reduction on Bone Metabolic Markers in Young Women"

NAME: _____

Inclusion Criteria - The inclusion criteria for this study require that each subject:

- ☐ Is an apparently healthy female
- ☐ Is 18-30 years of age.
- ☐ Is taking a combined oral contraceptive pill and has been for at least one month.
- ☐ Has not engaged in resistance exercise or moderate-high intensity aerobic exercise more than 2 times per week for the last 3 months.
- ☐ Is free of any orthopedic problems/injuries limiting exercise ability

Exclusion Criteria - The exclusion criteria for this study require that each subject:

- ☐ is not taking medications known to alter bone density or metabolism (other than a combined oral contraceptive pill).
- ☐ is not a current tobacco user (smoker/chewer).
- ☐ does not weigh more than 300 pounds and is not longer than 197.6 cm (77.8 in) or wider than 60.0 cm (23.6 in).
- ☐ does not have any orthopedic disabilities or injuries preventing them from participation in physical testing.
- ☐ Not be pregnant or think they may be pregnant
- ☐ Must not be taking any prescription medication other than birth control pill
- ☐ Must not have a history of cardiovascular or thromboembolism disease
- ☐ Must not be a current student of Dr. Debra Bemben
- ☐ Not be identified as moderate to high risk individuals as described by the American College of Sports Medicine: Must not have more than one of the following:
  - ☐ Father, Mother, sister, or brother that has had a sudden death before 55 years of age
  - ☐ Is a current cigarette smoker or within the last 6 months
  - ☐ Has been diagnosed with hypertension or is on hypertensive medication
  - ☐ Is on lipid-lowering medication or has a total cholesterol above 200mg/dL
  - ☐ Has confirmed fasting blood glucose of 100 mg/dL or above
  - ☐ Is clinically obese (BMI $\geq$ 30)

☐ Not have more than one of the following risk factors for Thromboembolisms

☐ Is clinically obese (BMI ≥ 30)
☐ Diagnosed with Crohn's or Inflammatory Bowel Disease
☐ Past fracture of a hip, pelvis, or femur
☐ Major Surgery within the last 6 months
☐ Have varicose veins
☐ Family history of Deep Vein Thrombosis or Pulmonary Embolism

NOTES: _____

I, _____, screened the above potential research participant. Based on the above criteria, this individual [☐ qualifies/ ☐ does not qualify] for the research study.

PI's Signature: _____

Appendix C

Informed Consent and Authorization to Use or Disclose Protected Health Information

Version 09/08/10 *IRB No: 16049*

Consent Form
University of Oklahoma Health Sciences Center (OUHSC)
University of Oklahoma – Norman Campus

Effects of 12 Weeks Walking with or without Blood Flow Reduction on Bone Metabolic Markers in Young Women

Sponsor: American College of Sports Medicine

Principal Investigator: **Debra Bemben, PhD**
University of Oklahoma
405-325-5211

This is a research study. Research studies involve only individuals who choose to participate. Please take your time to make your decision. Discuss this with your family and friends.

Why Have I Been Asked To Participate In This Study?
You are being asked to take part in this trial/study because you meet the inclusion criteria of a female between the ages of 18-30 years who has not exercised more than twice per week for the last 3 months and is currently taking a combined oral contraceptive pill.

Why Is This Study Being Done?
The purpose of this study is to investigate the effects of 12 weeks of low-intensity treadmill walking with blood flow reduction on bone metabolism and bone health. It is unknown if reducing blood flow during treadmill walking will benefit bone health, muscle size and strength, and aerobic fitness in young women.

What is the Status of the Devices or Procedures involved in this study?
No experimental devices or procedures will be used in this research study.

How Many People Will Take Part In The Study?
About 45 young women will take part in this study, all at this location.

What Is Involved In The Study?
If you take part in this study, you will have the following tests and procedures:

Consent Form and Questionnaires
You will be asked to thoroughly review, and then sign and date an informed consent document (this document) and additional screening forms (health history questionnaire, physical activity readiness, and menstrual history questionnaire) to determine your eligibility to participate in this study. You may be excluded from the study if any of your questionnaire responses indicate that you have an exclusion criterion for the study. (30 minutes or as long as needed)

APPROVED
SEP 16 2011
OUHSC IRB

APPROVAL EXPIRES
JUL 31 2012
OUHSC IRB

Randomization:
If you are eligible to participate, you will be randomized into one of three study groups. Randomization means that you are put in a group by chance (like rolling dice). The three groups are low-intensity treadmill walking control group (CON-WALK), low-intensity treadmill walking with blood flow reduction group (BFR-WALK), or a non-exercise control group (CON). The CON-WALK and BFR-WALK groups will walk on a treadmill 4 times/week for 12 weeks, with each exercise session separated by at least 24 hrs. Exercise intensity will be set at a treadmill speed that is moderately difficult. The resultant exercise speeds will range between 3.0-3.5 mph for most subjects. The treadmill will be flat for the first 4 wks of training and then elevated to a 1% incline for training wks 5-8. The incline will then be increased to a 2% incline for wks 9-12.

Before each session begins, the BFR-WALK participants will be seated and fitted with electronically controlled and monitored elastic pressure cuffs which will be placed on the upper most portions of their legs and inflated to a lower pressure for 30 seconds and then deflated. This process will be repeated by adding more pressure until the target exercise pressure is reached. This process of slowly reaching the exercise pressures will take approximately 3-5 min. The initial exercise pressures will be kept for the first 6 wks of the training program. During the last 6 wks of training, the exercise pressures will increased.

Similar to the exercise pressures being adjusted over the initial portion of the training program, exercise duration will also be adjusted. During the first week, each exercise session will be 10 minutes in duration. At the start of week two, session duration will increase to 15 min, then, at the start of wk three, duration will increase to 20 min and it will remain constant for the remainder of the study.

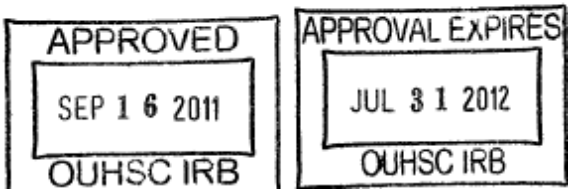
The CON group will be asked not to change their current physical activity levels for the duration of the training period and will only participate in the baseline and post-training testing sessions

All Three Groups will have the following tests done:

Height and Weight
Your height and weight will be measured.

Peripheral Quantitative Computed Tomography (pQCT) scans:
You will have 3 pQCT scans, a type of x-ray, of the right lower leg and one scan of the right thigh to measure muscle area (MCSA) and bone quantity and quality. The pQCT scan is a simple, non-invasive procedure where you will sit as still as possible in a chair with your leg in a positioning brace for approximately 15 minutes. Scans will be administered at the beginning and at the end of the 12 week training period.

Dual Energy X-ray Absorptiometry (DXA) Testing:
You will complete a full body, both hips, and lower spine DXA scans, a type of x-ray, to determine body composition (fat mass, lean tissue mass) and bone density. The total



series of scans will take about 20 minutes. You will have these scans at the beginning and at the end of the 12 week training period.

One repetition maximum (1RM) testing:
You will perform 1RM testing to determine muscular strength of the muscles that flex and extend the lower leg using the knee flexion and knee extension weight machines in the Neuromuscular Laboratory. Prior to the test, you will perform two warm-up sets of 8-10 reps at a light load (estimated 50% 1RM). Following warm-up, you will complete one repetition of a given weight through the full range of motion. The weight is increased until you cannot complete the repetition, and this will be achieved within 5 attempts. The greatest weight lifted through the full range of motion will be considered the 1RM. Two minutes of recovery will be allowed between attempts. This test will be done at the beginning and end of the 12 week training period. (20-25minutes)

Maximal Voluntary Contraction (MVC) testing:
You will be asked to flex and extend your lower leg against an immovable object as hard as possible. You will perform one repetition for each muscle group at 50% of maximal perceived effort. After this initial warm-up, you will be asked to perform three repetitions at maximal effort for the knee flexor and extensor muscles. This test will be done at the beginning and end of the 12 week training period. (15 minutes)

Maximal Graded Exercise Test (GXT):
You will undergo a maximal graded exercise test to determine your aerobic capacity and maximal heart rate. A sterile mouthpiece attached to a head harness will be secured on your head. You will then have a nose clip placed on your nose. The mouthpiece will direct your breath through a machine that will measure your aerobic capacity. You will be asked to walk on a treadmill. The treadmill speed will then be increased gradually as a warm up. After warm up, the speed will be increased to 5.5 mph and held constant for the remainder of the test. Every two minutes thereafter, the treadmill grade will be increased 2%. You will be encouraged to exercise until you feel like you cannot continue, but you may stop the test at any time. This will take about 20 minutes and will be completed before and after the 12 week training period.

Blood Sampling:
You will be asked to give a blood sample (approximately 5 tsp), following an 8-hour overnight fast. Your blood sample will be kept for at least 2 years in the Bone Density Laboratory in case samples have to be reanalyzed. Thereafter, the blood will be discarded. Your blood sample will be used to measure bone metabolic markers as well as the hormones cortisol and IGF-1. Bone metabolic markers indicate the activity of the normal processes of bone breakdown and bone formation. Cortisol levels are used as a marker of stress and IGF-1 is important for normal growth and repair of the body's tissues. You will be asked to donate a total of two blood samples, one at the beginning and one at the end of the 12 week training period. This procedure will take about 5 minutes.

Pregnancy Testing:

APPROVED
SEP 1 6 2011
OUHSC IRB

APPROVAL EXPIRES
JUL 3 1 2012
OUHSC IRB

You will be asked to provide a urine sample for a pregnancy test before having a DXA or pQCT scan.

3-Day Nutrition Logs:
To determine habitual nutritional intake, you will be asked to complete a 3-day dietary log, consisting of two weekdays and one weekend day, during week 1 and week 12. It is important to monitor dietary habits during this study. You will be encouraged to keep your dietary intakes constant for the duration of the study.

How Long Will I Be In The Study?
Your participation is anticipated to last approximately fourteen (14) weeks. During the first week, you will have a total of four visits. If you are randomized into one of the two exercise groups, you will be asked to visit the Neuromuscular Laboratory 4 times per week over the next 12 weeks. During the 14th week, you will have 3 visits to finish all post-training testing.

There may be anticipated circumstances under which your participation may be terminated by the investigator without regard to your consent, for reasons such as not adhering to all study guidelines, for health concerns observed by the investigators, or if the study is terminated by the Investigators.

You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher first.

What Are The Risks of The Study?
While in the study, you are at risk for these side effects. There may also be risks that are currently unforeseeable. You should discuss these with the researcher prior to providing your consent to participate.

Risks and side effects related to blood flow restriction:
- Feeling faint, fatigued, lightheaded and possibility of passing out.
- Bruising and discomfort caused by the strap.
- Because you are taking combined oral contraceptives, you are at increased risk for a blood clot. Due to the nature of the blood flow reduced exercise, there may be increased risk for deep vein thrombosis or venous thromboembolism. However, neither an acute (single bout) nor chronic training period (4 weeks) of BFR exercise showed an increased risk of blood clotting.

Risks and side effects related to exercise and exercise testing:
- Feeling faint, fatigued, lightheaded and possibility of passing out due to physical exertion.
- Muscle soreness and/or stiffness beginning within 24 hours post-exercise and lasting for several days.
- Muscle fatigue, shortness of breath, and elevated heart rate during and trouble walking immediately following maximal exercise tests.

Risks and side effects related to having a blood draw:
- Bleeding at the sight of puncture
- Pain at the sight of puncture

APPROVED
SEP 16 2011
OUHSC IRB

APPROVAL EXPIRES
JUL 31 2012
OUHSC IRB

- Bruising to the surrounding area for a couple of days
- Feeling lightheaded or faint
- A slight possibility of infection which can occur anytime the skin is broken (rare)

Risks and side effects related to having a pQCT and DXA scan:

- This research study involves exposure to radiation from 8 DXA scans and 8 pQCT scans, which are types of x-ray procedures. These procedures are for research only and not needed for your medical care. The amount of additional radiation to which you will be exposed is approximately 1% of the amount of radiation to which we are exposed annually from background sources such as the Earth and Sun. In addition to any radiographic procedures that are being done as part of this research, you may also be exposed to radiation from procedures that are part of your normal care. The risk from radiation exposure increases over your lifetime as you receive additional exposure to radiation.

Risks for Females:

- If you are a female, you must not be and should not become pregnant nor breast-feed an infant while in this study. Undergoing a particular procedure or treatment involved in this study while pregnant or breastfeeding may involve risks to an embryo, fetus or infant, including birth defects which are currently unforeseeable. Because the use of a combined oral contraceptive pill is one of the inclusion criteria, you should inform the PI or Co-PI if you stop taking or change your method of birth control. If you suspect that you are pregnant while on this study, you will be withdrawn until a pregnancy test has been taken. If pregnancy is confirmed, you will be permanently withdrawn from the study.

Are There Benefits to Taking Part in The Study?

If randomly assigned to an exercise group, you will receive the general health benefits associated with a regular exercise program.

What Other Options Are There?

There are no alternative procedures for this investigation; your alternative is to not participate.

What About Confidentiality?

Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the faculty members and graduate students appointed to this protocol from the Department of Health & Exercise Science at the University of Oklahoma, and the OUHSC Institutional Review Board.

What Are the Costs?

There is no cost to you for participating in this study.

APPROVED
SEP 16 2011
OUHSC IRB

APPROVAL EXPIRES
JUL 31 2012
OUHSC IRB

Will I Be Paid For Participating in This Study?
If you are assigned to one of the two exercise groups, you will receive $80 upon full completion of the study. If you participate in the pre-testing session, you will receive a reduced compensation of $10. If you finish at least 24 exercise sessions, you will receive a reduced compensation of $20. If you complete the pre-testing sessions and all of the exercise sessions but withdrawal before completing the post-testing sessions, you will receive a reduced compensation of $40.

If you are assigned to the non-exercise control group, you will receive a total compensation of $30 upon full completion. If you withdrawal before finishing the post-testing sessions, you will receive a reduced compensation of $15.

What if I am Injured or Become Ill While Participating in this Study?
In the case of injury or illness resulting from this study, emergency medical treatment will be available. If injury occurs as a result of participation, you should consult with your personal physician to obtain treatment. However, you or your insurance company will be responsible for the costs associated with this treatment. No funds have been set aside by The University of Oklahoma Health Sciences Center or the Department of Health & Exercise Science to compensate you or pay for the costs associated with treatment in the event of injury.

What Are My Rights As a Participant?
Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. However, please be sure to discuss leaving the study with the principal investigator or your regular physician. You may discontinue your participation at any time without penalty or loss of benefits, to which you are otherwise entitled.

We will provide you with any significant new findings developed during the course of the research that may affect your health, welfare or willingness to continue your participation in this study.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished and you consent to this temporary restriction.

Whom Do I Call If I have Questions or Problems?
If you have questions, concerns, or complaints about the study or have a research-related injury, contact Debra Bemben, PhD at 405-325-5211 or Kaelin Young at 253-255-5617.

If you cannot reach the Investigator or wish to speak to someone other than the investigator, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

APPROVED
SEP 16 2011
OUHSC IRB

APPROVAL EXPIRES
JUL 31 2012
OUHSC IRB

For questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

Signature:
By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

PARTICIPANT SIGNATURE (age ≥18)	Printed Name	Date
SIGNATURE OF PERSON OBTAINING CONSENT	Printed Name	Date

IRB Office Version Date: 07/07/2009

APPROVED
SEP 1 6 2011
OUHSC IRB

APPROVAL EXPIRES
JUL 3 1 2012
OUHSC IRB

AUTHORIZATION TO USE or DISCLOSE
PROTECTED HEALTH INFORMATION FOR RESEARCH

An additional Informed Consent Document for Research Participation may also be required.
Form 2 must be used for research involving psychotherapy notes.

Title of Research Project: **Effects of 12 Weeks Walking with or without Blood Flow Reduction on Bone Metabolic Markers in Young Women**

Leader of Research Team: **Debra Bemben, PhD**

Address: **1401 Asp Avenue HHC #119, Norman, OK 73019**

Phone Number: **405-325-5211**

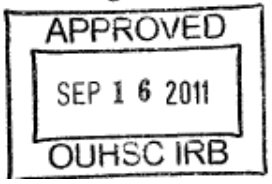
If you decide to join this research project, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share (disclose) information about you that is considered to be protected health information for their research. Protected health information will be called private information in this Authorization.

Private Information To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your private information. If you give permission, the researchers may use or share with the people identified in this Authorization any private information related to this research from your medical records and from any test results. Information, used or shared, may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form, medical records and charts, name, address, telephone number, date of birth, race, and government-issued identification number.

Purposes for Using or Sharing Private Information. If you give permission, the researchers may use your private information to investigate the effect of blood flow reduction on bones and muscle tissue in young women

Other Use and Sharing of Private Information. If you give permission, the researchers may also use your private information to develop new procedures or commercial products. They may share your private information with the research sponsor, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS). The researchers may also share your private information with all researchers working on this project or listed as a co-investigator.

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information





based on this authorization could re-release the information to others and federal law would no longer protect it.

YOU MUST UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING ANY CONDITIONS CONSIDERED AS A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT ARE NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA, AND HUMAN IMMUNODEFICIENCY VIRUS ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS).

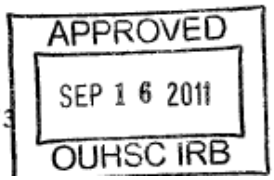
Voluntary Choice. The choice to give OUHSC researchers permission to use or share your private information for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your private health information if you want to participate in the research and if you revoke your authorization, you can no longer participate in this study.

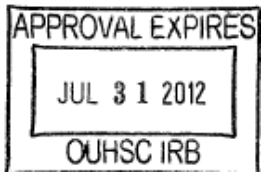
Refusing to give permission will not affect your ability to get routine treatment or health care from OUHSC.

Revoking Permission. If you give the OUHSC researchers permission to use or share your private information, you have a right to revoke your permission whenever you want. However, revoking your permission will not apply to information that the researchers have already used, relied on, or shared.

End of Permission. Unless you revoke it, permission for OUHSC researchers to use or share your private information for their research will never end. You may revoke your permission at any time by writing to:

Privacy Official
University of Oklahoma Health Sciences Center
PO Box 26901, Oklahoma City, OK 73190
If you have questions call: (405) 271-2511





Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by Debra Bemben, PhD, permission to share your private information for the research project called Effects of 12 Weeks Walking with or without Blood Flow Reduction on Bone Metabolic Markers in Young Women.

Patient/Subject Name: _____

_____ _____
Signature of Patient-Subject or Parent if subject is a child — Date

Or

_____ _____
Signature of Legal Representative** — Date

**If signed by a Legal Representative of the Patient-Subject, provide a description of the relationship to the Patient-Subject and the Authority to Act as Legal Representative:

_____
OUHSC may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Patient-Subject or the Legal Representative at the time this signed form is provided to the researcher or his representative.

IRB No.: 16049

APPROVED
SEP 16 2011
OUHSC IRB

APPROVAL EXPIRES
JUL 31 2012
OUHSC IRB

Appendix D

Study Questionnaires

Bone Density Research Laboratory
Department of Health and Exercise Science
University of Oklahoma

MENSTRUAL HISTORY QUESTIONNAIRE

Subject ID: _____ Date: _____

We are asking you to give us as complete a menstrual history as possible. All information you provide will be strictly confidential.

Are you pregnant? (circle your response below)

YES – Do not complete the rest of this form.

NO – Complete sections A and B of this form.

SECTION A: CURRENT MENSTRUAL STATUS

1. Approximately how many menstrual periods have you had during the past 12 months?

2. Circle the months in which your period occurred. This means from this time last year until the present month.

   JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC

3. What is the usual length of your menstrual cycle (first day menses to first day next menses)

   ____ days. Today is day _____ of your present menstrual cycle.

4. What was the date of your last period?

5. When do you expect your next menstrual period?

6. What is the length (number of days) of your menstrual flow on the average?

How many of these days would you term "heavy"

7. Do you experience cramps during menstruation (dysmenorrhea)? If yes, how many days does this last?

8. Do you experience symptoms of premenstrual syndrome (i.e., weight gain, increased eating, depression, headaches, anxiety, breast tenderness)? If yes, list the symptoms.

9. Do you take oral contraceptives or any other medication that includes estrogen and/or progesterone? If no, skip to question 10.

   If yes, how long have you been taking the birth control pill?

   What is the brand name and dosage of the oral contraceptive you are taking?

   Has the pill affected your menstrual cycle (regularity, length and amount of flow, length of cycle)? If yes, indicate changes.

10. Have you taken oral contraceptives in the past? If no, skip to SECTION B.

    If yes, what was the brand name and dosage?

    When did you start taking the pill; for how long; and when did you stop taking it?

11. If you answered yes to 9 or 10, did you experience a weight gain and/or a change in appetite as a result of oral contraceptive use? If so, please indicate amount of weight gain.

12. If you are perimenopausal, are you experiencing menopausal symptoms? Please list your symptoms (i.e., hot flushes, mood swings, headaches etc.)

SECTION B: PAST MENSTRUAL HISTORY

1. At what age did you experience your **first** menstrual period?

2. Were your periods regular (occurring monthly) during the first two years after menstruation began? If no, at what age did your periods eventually become regular?

3. Did you perform any form of athletic training prior to your first menstrual period? If yes, indicate type of training (i.e., gymnastics, track, basketball, etc.) and the number of years you trained for each activity.

4. Has there been any time in the past where your periods were irregular or absent? If no, skip to question 5.

   If yes, did these periods coincide with unusual bouts of training, or with a period of stress?
   How long did this occur?

5. Have you ever consulted a doctor about menstrual problems (specifically, about irregular or missing periods)? If no, skip to question 6.

   If yes, have you ever been diagnosed as having a shortened luteal phase?

   Have you ever been tested to determine if you were ovulating normally?

6. Have you ever consulted a physician about any problems relating to your hormonal system? If so, please explain.

Bone Density Research Laboratory

OU Department of Health and Exercise Science

Health Status Questionnaire

Instructions Complete each question accurately. All information provided is confidential.

(NOTE: The following codes are for office use only: RF; MC; SLA; SEP)

Part 1. Information about the individual

1._____
Date

2. _____
Legal name Nickname

3._____
Mailing address

_____
Home phone Business phone

4.Gender (circle one): Female Male (RF)

5. Year of birth: _____ Age _____

6. Number of hours worked per week: Less than 20 20-40 41-60 Over 60

(SLA) More than 25% of time spent on job (circle all that apply)

Sitting at desk Lifting or carrying loads Standing Walking Driving

Part 2. Medical history

7. (RF) Circle any who died of heart attack before age 50:

Father Mother Brother Sister Grandparent

8.Date of: Last medical physical exam: _____Last physical fitness test: _____
Year Year

9. Circle operations you have had:

Back (SLA)	Heart (MC)	Kidney (SLA)	Eyes (SLA)	Joint (SLA)	Neck (SLA)
Ears (SLA)	Hernia (SLA)	Lung (SLA)	Other _____		

10. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

Alcoholism (SEP)	Diabetes (SEP)	Kidney problem (MC)
Anemia, sickle cell (SEP)	Emphysema (SEP)	Mental illness (SEP)
Anemia, other (SEP)	Epilepsy (SEP)	Neck strain (SLA)
Asthma (SEP)	Eye problems (SLA)	Obesity (RF)
Back strain (SLA)	Gout (SLA)	Osteoporosis
Bleeding trait (SEP)	Hearing loss (SLA)	Phlebitis (MC)
Bronchitis, chronic (SEP)	Heart problems (SLA)	Rheumatoid arthritis (SLA)
Cancer (SEP)	High blood pressure (RF)	Stroke (MC)
Cirrhosis, liver (MC)	Hypoglycemia (SEP)	Thyroid problem (SEP)
Concussion (MC)	Hyperlipidemia (RF)	Ulcer (SEP)
Congenital defect (SEP)	Infectious mononucleosis (MC)	Other _____

11. Circle all medicine taken in last 6 months:

Blood thinner (MC)	Epilepsy medication (SEP)	Nitroglycerin (MC)
Diabetic pill (SEP)	Heart-rhythm medication (MC)	Estrogen
Digitalis (MC)	High-blood-pressure medication (MC)	Thyroid
Diuretic (MC)	Insulin (MC)	Corticosteroids
Asthma	Other _____	

12. Any of these health symptoms that occurs frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

1 = Practically never 2 = Infrequently 3 = Sometimes 4 = Fairly often 5 = Very often

a. Cough up blood (MC)	d. Leg pain (MC)	g. Swollen joints (MC)
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
b. Abdominal pain (MC)	e. Arm or shoulder pain (MC)	h. Feel faint (MC)
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
c. Low back pain (SLA)	f. Chest pain (RF) (MC)	I. Dizziness (MC)
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
j. Breathless with slight exertion (MC)		
1 2 3 4 5		

13. Do any of the following apply:

- A sudden death in your biological father or brother, or mother or sister prior to age 55 or 65, respectively? Yes No
- Current smoker or have you quit smoking within the past 6 months? Yes No
- Do you take hypertensive medication or have a confirmed systolic or diastolic blood pressure ≥140 or 90 mmHg, respectively? Yes No
- Take lipid lowering medication or have high blood cholesterol? Yes No
- You have a confirmed fasting blood glucose of ≥100 mg/dL? Yes No
- Have you recently been diagnosed as clinically obese (BMI > 30)? Yes No
- Are you sedentary? Yes No
- Diagnosed Crohn's or Inflammatory Bowel Disease Yes No
- Past fracture of a hip, pelvis, or femur Yes No
- Major Surgery within the last 6 months Yes No
- Been diagnosed with varicose veins Yes No
- Family history of Deep Vein Thrombosis or Pulmonary Embolism Yes No

Part 3. Health-related behavior

14. (RF) Do you now smoke or chew tobacco? Yes No

15. If you are a smoker, indicate number smoked per day:

Cigarettes:	40 or more	20-39	10-19	1-9
Cigars or pipes only:	5 or more or any inhaled		Less than 5, none inhaled	

16. Weight now: _____lb. One year ago: _____lb.. Age 21: _____lb.

17. Thinking about the things you do at work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

    1. Much more active
    2. Somewhat more active
    3. About the same
    4. Somewhat less active
    5. Much less active
    6. Not applicable

18. Now, thinking about the things you do outside of work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

    1. Much more active

2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

19. Do you regularly engage in strenuous exercise or hard physical labor?

1. Yes (answer question # 19) 2. No (stop)

20. Do you exercise or labor at least three times a week?

1. Yes 2. No

Bone Density Research Laboratory
OU Department of Health and Exercise Science
3-Day Dietary Log

Subject ID_____
Date_____

Instructions:

Please record everything that you eat for **two days during the week** and **one day during the weekend**. Include the food/drink item with brand names if applicable, the amount ingested (serving size), and method of preparation (baked, fried etc), if applicable. Please be sure to include all beverages including protein/ meal replacements and alcoholic beverages. Please be as specific as possible.

Serving Size Handy Guide: See Attached Appendix

Day 1 during the week: _____

Meal/Time	Food/Drink	Amount (1 cup, 8 oz, number of slices, etc.)	How Prepared (fried, baked, etc.)
Breakfast			
Snack			
Lunch			
Snack			
Dinner			
Snack			

Day 2 during the week: _____

Meal/Time	Food/Drink	Amount (1 cup, 8 oz, number of slices, etc.)	How Prepared (fried, baked, etc.)
Breakfast			
Snack			
Lunch			
Snack			
Dinner			
Snack			

Day 3 during the weekend: _____

Meal/Time	Food/Drink	Amount (1 cup, 8 oz, number of slices, etc.)	How Prepared (fried, baked, grilled, etc.)
Breakfast			
Snack			
Lunch			
Snack			
Dinner			
Snack			

Appendix E

IRB Approval Letter

The University of Oklahoma®
Health Sciences Center
INSTITUTIONAL REVIEW BOARD

IRB Number: 16049
Meeting Date: August 15, 2011
Approval Date: September 16, 2011

September 23, 2011

Debra Bemben, Ph.D.
Univ of Oklahoma, Dept of Health & Exercise Sci
1401 Asp Avenue
Norman, OK 73069

RE: Effects of 12 Weeks Walking With and Without Blood Flow Reduction on Bone Metabolic Markers in Young Women

Dear Dr. Bemben:

The University of Oklahoma Health Sciences Center's Institutional Review Board (IRB) reviewed the above-referenced research protocol at its regularly scheduled meeting on August 15, 2011. It is the IRB's judgement that the rights and welfare of the individuals who may be asked to participate in this study will be respected; that the proposed research, including the process of obtaining informed consent, will be conducted in a manner consistent with the requirements of 45 CFR 46 or 21 CFR 50 & 56, as amended; and that the potential benefits to participants and to others warrant the risks participants may choose to incur.

On behalf of the IRB, I have verified that the specific changes requested by the convened IRB have been made. Therefore, on behalf of the Board, I have granted final approval for this study.

This letter documents approval to conduct the research as described:

Grant Application Dated: April 01, 2011
Consent form - Subject Dated: September 08, 2010
Priv - Research Auth 1 Dated: January 06, 2005
Recruitment flyer Dated: August 01, 2011
Advertisement - Email Dated: August 01, 2011
Phone Script - Recruitment Dated: August 01, 2011
Protocol Dated: September 01, 2009
Other Dated: August 01, 2011 Health Status and Menstrual History questionnaires
Other Dated: August 01, 2011 PAR-Q & YOU
Other Dated: August 01, 2011 3-Day Dietary Log with serving size choices
Other Dated: August 01, 2011 Daily Exercize Log

As principal investigator of this protocol, it is your responsibility to make sure that this study is conducted as approved by the IRB. Any modifications to the protocol or consent form, initiated by you or by the sponsor, will require prior approval, which you may request by completing a protocol modification form.

It is a condition of this approval that you report promptly to the IRB any serious, unanticipated adverse events experienced by participants in the course of this research, whether or not they are directly related to the study protocol. These adverse events include, but may not be limited to, any experience that is fatal or immediately life-threatening, is permanently disabling, requires (or prolongs) inpatient hospitalization, or is a congenital anomaly, cancer or overdose. For multi-site protocols, the IRB must be informed of serious adverse events at all sites.

The approval granted expires on July 31, 2012. Should you wish to maintain this protocol in an active status beyond that date, you will need to provide the IRB with an IRB Application for Continuing Review (Progress Report) summarizing study results to date. The IRB will request a progress report from you approximately three months before the anniversary date of your current approval.

If you have questions about these procedures, or need any additional assistance from the IRB, please call the IRB office at (405) 271-2045 or send an email to irb@ouhsc.edu. Finally, please review your professional liability insurance to make sure your coverage includes the activities in this study.

Sincerely yours,

Karen J. Beckman, M.D.
Chair, Institutional Review Board

Post Office Box 26901 • 1000 S.L. Young Blvd., Room 176
Oklahoma City, Oklahoma 73126-0901 • (405) 271-2045 • FAX: (405) 271-1677

Appendix F

Exercise Protocol

Week	Time to set exercise pressure	Exercise Pressure (mmHg)	Treadmill Elevation	Treadmill Speed (mph)	Exercise Duration
Wk 1	~3 min	~140	0%	3.0 – 3.8	10 min
Wk 2	~3 min	~140	0%	3.0 – 3.8	15 min
Wk 3	~3 min	~140	0%	3.0 – 3.8	20 min
Wk 4	~3 min	~140	0%	3.0 – 3.8	20 min
Wk 5	~3 min	~160	1%	3.0 – 3.8	20 min
Wk 6	~3 min	~160	1%	3.0 – 3.8	20 min
Wk 7	~5 min	~160	1%	3.0 – 3.8	20 min
Wk 8	~5 min	~160	1%	3.0 – 3.8	20 min
Wk 9	~5 min	~180	2%	3.0 – 3.8	20 min
Wk 10	~5 min	~180	2%	3.0 – 3.8	20 min
Wk 11	~5 min	~180	2%	3.0 – 3.8	20 min
Wk 12	~5 min	~180	2%	3.0 – 3.8	20 min

Appendix G

Raw Data

	Group	age	HtM	PreWt	postWt	preBMI
1	1.00	19.00	1.57	54.70	60.60	22.30
2	1.00	22.00	1.67	71.90	72.20	25.90
3	1.00	21.00	1.64	53.60	53.20	19.90
4	1.00	22.00	1.63	74.50	75.70	28.00
5	1.00	27.00	1.75	62.50	61.00	20.40
6	1.00	19.00	1.60	62.40	63.40	24.40
7	1.00	22.00	1.69	73.20	75.90	25.80
8	1.00	20.00	1.70	63.60	68.20	22.10
9	1.00	21.00	1.73	65.50	66.70	21.90
10	1.00	22.00	1.70	57.70	57.40	20.00
11	2.00	19.00	1.76	66.90	66.20	21.60
12	2.00	21.00	1.64	44.00	42.50	16.50
13	2.00	20.00	1.60	54.80	54.00	21.50
14	2.00	22.00	1.68	62.60	60.50	22.30
15	2.00	21.00	1.66	52.90	53.20	19.20
16	2.00	19.00	1.62	55.90	55.50	21.30
17	2.00	21.00	1.60	54.00	54.40	21.10
18	2.00	26.00	1.79	63.30	64.80	19.80
19	2.00	19.00	1.64	64.20	62.60	24.00
20	2.00	19.00	1.65	67.90	68.40	25.10
21	2.00	19.00	1.68	56.00	55.70	19.80
22	3.00	23.00	1.65	55.20	56.20	20.40
23	3.00	20.00	1.65	58.20	57.00	21.40
24	3.00	19.00	1.66	65.90	66.00	24.10
25	3.00	20.00	1.55	52.60	51.10	22.00
26	3.00	22.00	1.70	69.70	69.70	24.10
27	3.00	21.00	1.59	54.10	53.50	21.40
28	3.00	20.00	1.68	67.20	69.00	24.00
29	3.00	21.00	1.68	60.80	59.00	21.70
30	3.00	18.00	1.73	73.50	74.30	24.70
31	3.00	25.00	1.72	61.90	63.50	21.00

	PostBMI	PreBF	postBF	PctChgBF	prebflbm	postbflm
1	24.70	25.20	33.40	32.50	35.60	35.30
2	26.00	46.30	47.00	1.50	35.10	34.50
3	19.80	27.20	28.50	4.80	36.00	35.70
4	28.50	47.60	44.20	-7.10	35.60	39.20
5	19.90	34.10	33.30	-2.30	38.20	37.90
6	24.80	30.30	32.10	5.90	38.10	37.70
7	26.70	41.90	43.00	2.60	39.40	40.10
8	23.70	31.60	32.40	2.50	40.30	43.20
9	22.30	25.10	27.60	10.00	45.90	44.90
10	19.90	27.20	26.40	-2.90	39.20	39.30
11	21.40	32.70	31.90	-2.40	39.20	39.20
12	15.90	21.40	21.80	1.90	31.90	30.60
13	21.20	26.50	26.40	-.40	37.00	36.80
14	21.60	32.70	32.20	-1.50	39.10	38.10
15	19.30	21.90	22.70	3.70	38.50	38.78
16	21.10	34.60	33.50	-3.20	31.90	32.00
17	21.30	28.80	28.70	-.30	35.60	36.10
18	20.20	27.30	28.20	3.30	43.10	43.50
19	23.40	37.80	36.50	-3.40	34.30	34.30
20	25.30	36.50	35.30	-3.30	37.70	41.20
21	19.70	24.10	23.50	-2.50	36.90	37.00
22	20.80	30.80	34.50	12.00	35.30	33.90
23	20.90	27.80	28.80	3.60	39.30	37.80
24	24.10	42.80	43.50	1.60	32.30	31.90
25	21.40	33.40	32.10	-3.90	32.40	32.10
26	24.10	34.40	34.60	.60	41.80	41.80
27	21.20	28.90	27.20	-5.90	35.60	36.20
28	24.60	34.70	36.20	4.30	40.70	40.50
29	21.00	24.70	24.90	.80	42.10	41.20
30	25.00	40.00	42.50	6.30	38.80	37.50
31	21.60	29.50	31.50	6.80	39.90	39.90

	PctChgbflbm	preFM	PtFM	PctChgFM	preleglm	postleglm
1	-.80	12.60	18.70	48.40	11.70	12.30
2	-1.70	32.90	33.40	1.50	12.50	11.90
3	-.80	14.10	14.90	5.70	12.10	12.20
4	10.10	35.10	33.10	-5.70	11.90	14.00
5	-.80	20.90	20.10	-3.80	13.80	13.80
6	-1.00	17.40	18.80	8.00	13.40	13.40
7	1.80	30.40	32.30	6.30	13.90	14.40
8	7.20	19.90	22.00	10.60	13.90	15.30
9	-2.20	16.40	18.20	11.00	16.50	15.80
10	.30	15.60	15.00	-3.80	13.70	13.50
11	.0	20.20	19.40	-4.00	14.00	14.30
12	-4.10	9.20	9.10	-1.10	10.40	10.00
13	-.50	14.10	14.00	-.70	12.30	12.00
14	-2.60	20.20	19.30	-4.50	13.20	13.10
15	.70	11.40	12.10	6.10	13.30	13.20
16	.30	17.80	17.00	-4.50	12.10	12.10
17	1.40	15.30	15.40	.70	11.90	11.90
18	.90	17.20	18.00	4.70	14.60	14.70
19	.0	22.20	21.00	-5.40	12.60	13.10
20	9.30	23.00	23.90	3.90	14.60	14.80
21	.30	12.40	12.00	-3.20	13.10	13.20
22	-4.00	16.80	19.10	13.70	11.60	11.01
23	-3.80	16.10	16.30	1.20	13.90	12.40
24	-1.20	25.90	26.40	1.90	12.00	11.90
25	-.90	17.40	16.30	-6.30	10.70	10.60
26	.0	23.80	23.90	.40	15.10	15.20
27	1.70	15.30	14.40	-5.90	11.70	11.80
28	-.50	23.10	24.60	6.50	14.30	13.90
29	-2.10	14.60	14.60	.0	15.00	14.60
30	-3.40	27.30	29.40	7.70	15.30	14.50
31	.0	18.00	19.90	10.60	12.30	12.50

	PctChgLegLM	prefnbmd	postfnbmd	prefnbmc	postfnbmc	preTrcbmd
1	5.10	1.07	1.07	4.69	4.64	.86
2	-4.80	1.06	1.08	5.00	5.08	.83
3	.80	.89	.91	3.94	3.99	.66
4	17.60	.91	.90	4.39	4.14	.63
5	.0	.95	.96	4.35	4.36	.78
6	.0	.98	1.00	4.50	4.62	.85
7	3.60	1.07	1.07	4.71	4.68	.80
8	10.10	1.13	1.12	5.38	5.35	.93
9	-4.20	1.20	1.23	5.83	5.83	1.09
10	-1.50	.91	.89	4.39	4.34	.65
11	2.10	1.12	1.08	5.00	4.97	.85
12	-3.80	.95	.95	4.02	4.01	.74
13	-2.40	.94	.94	4.18	4.19	.79
14	-.80	1.05	1.05	5.06	5.08	.77
15	-.80	.96	.95	4.61	4.49	.82
16	.0	.88	.89	4.07	4.14	.66
17	.0	.90	.91	3.98	3.98	.79
18	.70	.98	.98	4.46	4.48	.66
19	4.00	1.02	1.02	4.54	4.61	.78
20	1.40	1.16	1.15	5.32	5.32	.88
21	.80	.94	.92	4.74	4.66	.75
22	-5.10	1.00	1.01	4.31	4.27	.73
23	-10.80	1.11	1.11	4.87	4.80	.88
24	-.80	1.11	1.09	5.13	5.03	.87
25	-.90	1.08	1.08	4.67	4.74	.89
26	.70	1.09	1.10	5.07	5.06	.84
27	.90	.96	.94	4.52	4.56	.76
28	-2.80	1.09	1.10	5.07	5.32	.95
29	-2.70	1.15	1.14	4.86	4.77	.85
30	-5.20	1.13	1.18	4.82	4.99	.86
31	1.60	1.23	1.24	6.07	6.23	.88

	postTrcbmd	preTrcbmc	postTrcbmc	preTHbmd	postTHbmd	preTHbmc
1	.84	8.47	8.11	1.10	1.09	30.02
2	.84	8.80	8.90	1.09	1.10	31.70
3	.68	6.92	7.18	.89	.90	25.02
4	.62	5.75	5.56	.85	.84	23.78
5	.78	9.01	8.99	.93	.93	28.05
6	.85	10.28	10.38	1.04	1.04	31.45
7	.78	10.33	9.81	1.03	1.03	32.35
8	.93	10.79	10.98	1.18	1.18	35.96
9	1.09	13.91	13.94	1.22	1.24	39.65
10	.65	7.74	7.55	.86	.85	26.95
11	.85	10.61	10.70	1.07	1.07	33.89
12	.75	7.51	7.62	.97	.97	25.85
13	.79	8.02	7.68	.96	.97	27.17
14	.78	8.38	8.50	.98	.98	29.10
15	.82	10.96	10.65	1.05	1.05	32.43
16	.66	7.08	7.00	.87	.86	25.93
17	.79	7.67	7.89	.96	.96	26.66
18	.67	7.79	7.95	.87	.87	27.04
19	.77	8.82	8.62	1.04	1.04	29.82
20	.87	10.13	10.03	1.09	1.09	33.14
21	.76	10.94	11.23	.95	.96	32.30
22	.75	7.41	7.87	.95	.97	27.75
23	.90	9.74	9.83	1.07	1.07	31.36
24	.88	10.23	10.33	1.13	1.14	33.30
25	.90	8.66	8.79	1.11	1.12	29.48
26	.83	8.61	8.54	1.11	1.10	32.03
27	.78	8.87	9.27	.98	.98	29.84
28	.97	13.74	14.12	1.18	1.20	38.47
29	.87	10.89	10.80	1.08	1.09	33.29
30	.88	9.91	10.37	1.08	1.10	31.55
31	.88	11.13	11.07	1.19	1.19	37.98

	postTHbmc	preSpbmd	postSpbmd	preSpbmc	postSpbmc	PreMVC30F
1	27.27	1.27	1.26	46.19	36.21	89.00
2	31.90	1.14	1.15	45.50	44.50	77.00
3	25.34	1.07	1.09	39.67	40.26	58.00
4	23.43	1.01	1.01	36.22	36.27	87.00
5	27.94	1.11	1.11	44.96	44.80	76.00
6	32.03	1.35	1.35	52.05	52.46	81.00
7	31.89	1.16	1.17	51.19	52.59	97.00
8	36.33	1.23	1.24	55.53	54.77	108.00
9	39.97	1.30	1.26	56.61	56.18	104.00
10	26.61	1.17	1.21	57.44	57.99	72.00
11	34.06	1.38	1.39	67.04	67.63	71.00
12	25.89	1.02	1.02	38.53	38.84	48.00
13	27.05	1.24	1.24	51.66	51.22	65.00
14	29.30	1.07	1.05	45.28	41.37	82.00
15	32.04	1.13	1.11	46.29	45.45	75.00
16	25.79	1.25	1.25	50.97	51.59	72.00
17	27.09	1.14	1.19	42.14	45.32	69.00
18	27.09	1.09	1.09	49.87	50.50	86.00
19	29.78	1.37	1.35	57.61	56.68	80.00
20	32.89	1.25	1.20	57.75	56.18	102.00
21	32.60	1.10	1.12	47.14	48.56	71.60
22	28.25	1.22	1.22	49.66	50.40	63.00
23	31.53	1.21	1.16	50.74	50.04	63.00
24	33.59	1.24	1.25	54.35	55.09	78.00
25	29.92	1.26	1.23	44.60	44.97	74.00
26	31.97	1.41	1.41	60.68	58.93	90.00
27	31.10	1.34	1.35	54.03	55.70	72.00
28	38.88	1.40	1.40	58.72	59.98	118.00
29	33.52	1.38	1.35	56.74	58.27	62.00
30	32.43	.99	.97	35.67	35.70	91.00
31	37.96	1.49	1.52	77.39	78.20	89.00

	ptMVC30F	PctChgMVC3 0F	preMVC30E	ptMVC30E	PctChgMVC3 0E	PreMVC60F
1	89.00	.0	113.00	119.00	5.30	93.00
2	74.00	-3.90	127.00	125.00	-1.60	82.00
3	78.00	34.50	73.00	105.00	43.80	62.00
4	91.00	4.60	159.00	154.00	-3.10	67.00
5	80.00	5.30	107.00	114.00	6.50	79.00
6	86.00	6.20	106.00	113.00	6.60	75.30
7	90.00	-7.20	115.00	108.00	-6.10	84.00
8	101.00	-6.50	167.00	193.00	15.60	102.00
9	94.00	-9.60	127.00	148.00	16.50	93.00
10	75.00	4.20	91.00	103.00	13.20	71.00
11	77.00	8.50	122.00	151.00	23.80	64.00
12	50.00	4.20	92.30	106.00	14.80	53.60
13	76.00	16.90	95.00	115.00	21.10	70.00
14	85.00	3.70	115.00	120.00	4.30	82.00
15	85.00	13.30	106.00	115.00	8.50	75.00
16	70.00	-2.80	113.00	104.00	-8.00	65.40
17	75.00	8.70	102.00	102.00	.0	59.00
18	84.00	-2.30	130.00	149.00	14.60	87.00
19	83.00	3.80	107.00	120.00	12.10	76.00
20	109.00	6.90	151.00	179.00	18.50	100.90
21	73.00	2.00	146.30	144.00	-1.60	73.60
22	65.00	3.20	127.00	126.00	-.80	61.00
23	62.00	-1.60	142.00	87.00	-38.70	59.00
24	81.00	3.80	157.00	158.00	.60	65.00
25	68.00	-8.10	84.00	81.00	-3.60	67.00
26	95.00	5.60	115.00	142.00	23.50	91.00
27	75.00	4.20	89.00	91.00	2.20	75.00
28	120.00	1.70	142.00	141.00	-.70	98.00
29	61.00	-1.60	117.00	134.00	14.50	63.00
30	85.00	-6.60	148.00	155.00	4.70	88.00
31	94.00	5.60	110.00	115.00	4.50	86.00

	PtMVC60F	PctChgMVC6 0F	PreMVC60E	PtMVC60E	PctChgMVC6 0E	PreKnExt
1	74.00	-20.40	192.00	187.00	-2.60	59.70
2	75.00	-8.50	163.00	173.00	6.10	56.80
3	71.00	14.50	105.00	139.00	32.40	56.80
4	63.00	-6.00	198.00	187.00	-5.60	71.00
5	79.00	.0	205.00	214.00	4.40	59.70
6	65.00	-13.70	154.30	161.00	4.30	68.20
7	84.00	.0	158.00	156.00	-1.30	68.20
8	94.00	-7.80	234.00	259.00	10.70	73.90
9	90.00	-3.20	191.00	206.00	7.90	73.90
10	79.00	11.30	148.00	161.00	8.80	54.00
11	73.00	14.10	188.00	222.00	18.10	68.20
12	50.00	-6.70	126.80	131.00	3.30	42.60
13	76.00	8.60	127.00	166.00	30.70	54.00
14	88.00	7.30	182.00	196.00	7.70	54.00
15	83.00	10.70	155.00	197.00	27.10	62.50
16	73.00	11.60	179.50	169.00	-5.80	51.10
17	74.00	25.40	138.00	130.00	-5.80	51.10
18	81.00	-6.90	227.00	211.00	-7.00	56.80
19	68.00	-10.50	164.00	192.00	17.10	56.80
20	103.00	2.10	198.00	225.00	13.60	73.90
21	70.00	-4.90	205.40	221.00	7.60	59.70
22	62.00	1.60	164.00	184.00	12.20	59.70
23	58.00	-1.70	169.00	161.00	-4.70	62.50
24	71.00	9.20	180.00	192.00	6.70	45.50
25	65.00	-3.00	136.00	121.00	-11.00	56.80
26	95.00	4.40	182.00	210.00	15.40	73.90
27	68.00	-9.30	132.00	139.00	5.30	45.50
28	95.00	-3.10	213.00	218.00	2.30	76.70
29	68.00	7.90	160.00	190.00	18.80	68.20
30	102.00	15.90	227.00	207.00	-8.80	68.20
31	82.00	-4.70	186.50	180.00	-3.50	56.80

	PtKnExt	PctChgKnExt	PreKnFlx	PtKnFlx	PctChgKnFlx	PreVo2
1	59.70	.0	59.70	65.30	9.40	44.90
2	59.70	5.10	56.80	56.80	.0	30.00
3	59.70	5.10	54.00	51.10	-5.40	47.30
4	71.00	.0	73.90	73.90	.0	33.80
5	62.50	4.70	56.80	59.70	5.10	36.10
6	71.00	4.10	62.50	59.70	-4.50	38.50
7	68.20	.0	59.70	62.50	4.70	29.60
8	73.90	.0	71.00	73.90	4.10	41.20
9	73.90	.0	68.20	62.50	-8.40	43.70
10	51.10	-5.40	51.10	51.10	.0	37.50
11	73.90	8.40	62.50	59.70	-4.50	39.90
12	42.60	.0	39.80	45.50	14.30	40.60
13	56.80	5.20	56.80	62.50	10.00	35.20
14	51.10	-5.40	62.50	68.20	9.10	35.80
15	68.20	9.10	56.80	59.70	5.10	46.90
16	51.10	.0	54.00	59.70	10.60	35.60
17	51.10	.0	62.50	62.50	.0	40.40
18	65.30	15.00	59.70	59.70	.0	38.60
19	56.80	.0	62.50	62.50	.0	39.10
20	79.50	7.60	68.20	71.00	4.10	42.60
21	65.30	9.40	54.00	56.80	5.20	37.00
22	62.50	4.70	56.80	51.10	-10.00	32.90
23	56.80	-9.10	51.10	56.80	11.20	41.60
24	45.50	.0	51.10	51.10	.0	36.40
25	56.80	.0	56.80	56.80	.0	39.50
26	65.30	-11.60	68.20	68.20	.0	33.40
27	45.50	.0	39.70	39.70	.0	.
28	73.90	-3.70	71.00	73.90	4.10	39.60
29	68.20	.0	62.50	59.70	-4.50	43.20
30	68.20	.0	62.50	62.50	.0	33.60
31	56.80	.0	59.70	56.80	-4.90	38.50

	PtVo2	PctChgVo2	PreCal	PtCal	PrePRO	PtPRO
1	39.20	-12.70	1044.30	1182.00	46.20	60.70
2	31.40	4.70	1570.30	1577.30	52.00	56.50
3	44.60	-5.70	2393.60	1905.50	97.40	102.80
4	34.10	.90	1686.70	2224.00	66.00	62.10
5	36.80	1.90	1961.80	1983.60	65.60	42.40
6	36.30	-5.70	1668.50	1619.90	79.10	60.20
7	30.70	3.70	2534.00	1707.70	58.50	53.10
8	39.40	-4.40	2541.40	3064.40	92.40	95.70
9	43.00	-1.60	3172.50	2630.80	91.90	71.90
10	36.80	-1.90	1161.70	1498.30	42.70	42.60
11	40.60	1.80	1848.00	1623.00	65.50	74.10
12	49.50	21.90	2282.30	1843.90	123.90	59.40
13	35.40	.60	2428.10	1480.00	51.10	54.90
14	40.30	12.60	2454.00	2485.80	85.20	91.50
15	44.70	-4.70	2653.00	1389.00	100.50	46.00
16	35.10	-1.40	2603.30	2483.40	71.60	77.00
17	41.10	1.70	2167.70	1793.50	78.70	81.80
18	35.70	-7.50	1573.60	1421.60	39.80	41.40
19	37.10	-5.10	1533.30	1206.90	53.20	56.30
20	39.00	-8.50	1405.00	1076.70	44.00	56.70
21	34.20	-7.60	2788.40	1771.60	95.10	70.00
22	25.60	-22.20	2526.00	1551.70	54.50	52.40
23	38.20	-8.20	1669.20	1953.00	95.00	83.10
24	34.60	-4.90	1671.00	1501.80	59.70	44.40
25	39.50	.0	2303.90	1476.50	98.60	72.00
26	29.80	-10.80	1169.60	1511.90	53.50	60.80
27	.	.	2068.00	2107.00	59.60	61.80
28	37.30	-5.80	1676.90	1412.10	53.50	57.20
29	45.00	4.20	1473.00	1628.40	87.50	69.80
30	32.70	-2.70	2299.10	1935.90	79.30	75.30
31	34.40	-10.60	2072.60	2069.40	88.20	100.20

	PreCHO	PtCHO	PreFAT	PtFAT	PreVitD	PtVitD
1	156.80	132.20	30.00	45.30	18.60	1.20
2	191.00	180.50	38.00	44.60	.80	1.18
3	312.30	198.40	81.40	54.80	201.70	168.00
4	199.50	296.80	69.40	86.80	.70	33.20
5	257.30	259.60	55.20	47.20	30.00	16.70
6	241.10	249.20	48.10	44.80	.0	68.50
7	301.70	197.10	122.80	78.50	61.30	11.70
8	352.70	421.60	87.60	113.40	7.75	80.50
9	384.20	273.60	114.50	105.00	.0	.0
10	71.40	106.60	38.80	47.90	29.30	29.90
11	184.90	219.80	64.30	47.50	.0	26.70
12	261.70	267.10	83.10	73.50	143.30	.0
13	246.60	200.40	49.00	40.60	66.70	126.70
14	283.60	304.30	104.30	101.70	192.40	68.00
15	279.50	179.00	82.70	54.30	44.30	1.24
16	440.20	325.80	72.90	101.50	.0	.80
17	274.60	239.50	87.20	57.50	68.90	76.90
18	229.10	173.20	59.20	65.50	40.00	2.98
19	210.00	139.40	53.90	45.50	18.90	23.50
20	117.30	94.60	34.20	53.60	12.00	.0
21	329.40	212.40	126.30	74.80	.40	20.60
22	472.50	205.70	51.00	57.00	81.50	58.70
23	189.90	264.40	55.10	60.40	104.80	157.50
24	258.10	195.80	49.70	65.60	2.84	.0
25	253.80	157.90	99.80	64.00	216.70	161.00
26	151.50	170.50	38.80	57.10	51.50	.0
27	317.10	311.20	60.50	74.00	84.40	45.90
28	228.30	155.30	56.80	58.00	35.30	1.90
29	173.50	233.40	43.80	48.90	79.30	59.90
30	313.10	227.10	84.90	56.50	7.60	266.70
31	240.30	227.00	77.70	74.90	4.00	1.20

	PreCa2	PtCa2	BapPre	BapPost	BAPchg	TrapPre
1	360.40	368.40	21.25	21.33	.40	2.47
2	528.50	641.50	27.06	23.34	-13.80	1.54
3	976.20	1025.20	32.52	34.40	5.80	3.46
4	511.20	519.30	17.63	18.25	3.50	1.84
5	1066.90	451.70	29.79	31.86	6.90	3.40
6	579.00	390.70	25.86	26.91	4.10	3.91
7	882.20	494.40	38.60	35.85	-7.10	2.48
8	1106.20	1137.60	40.52	38.66	-4.60	3.93
9	714.30	882.20	26.32	30.08	14.30	1.40
10	215.70	210.20	20.82	22.93	10.20	2.16
11	704.40	381.20	27.88	25.66	-7.90	3.79
12	886.10	450.40	21.41	21.45	.20	1.91
13	677.70	683.30	16.79	15.94	-5.00	1.47
14	1142.20	857.30	30.72	31.93	3.90	3.74
15	475.30	471.00	31.86	29.71	-6.70	3.51
16	273.90	715.60	32.68	28.46	-12.90	2.73
17	679.60	576.70	18.63	18.63	.0	2.42
18	438.20	359.90	39.53	37.67	-4.70	4.00
19	339.40	405.50	42.82	38.60	-9.80	3.55
20	196.00	354.50	20.82	19.50	-6.30	1.83
21	794.60	582.20	42.53	36.15	-15.00	2.07
22	1871.60	319.00	24.04	22.76	-5.30	1.82
23	499.70	703.80	16.36	16.02	-2.10	2.98
24	576.00	275.80	31.86	28.54	-10.40	2.76
25	1553.20	975.00	32.09	29.83	-7.10	2.27
26	357.10	323.50	34.97	29.96	-14.30	1.70
27	402.90	605.80	51.17	49.28	-3.70	2.94
28	365.20	489.40	31.88	25.95	-18.60	2.52
29	797.40	731.10	30.80	28.33	-8.00	2.20
30	1206.50	1219.80	47.31	34.91	-26.20	2.94
31	666.30	421.80	46.41	45.56	-1.80	2.72

	TrapPost	TRAPchg	PreTibMCSA	PtTibMCSA	PctChgTibMC	Pre66TOTCn
1	2.37	-4.00	6407.68	6733.92	5.10	343.71
2	1.57	2.10	5644.96	5622.88	-.40	386.91
3	3.86	11.50	6271.36	6359.36	1.40	318.72
4	1.62	-11.50	5717.92	6005.92	5.00	339.35
5	3.55	4.50	6735.20	6949.12	3.20	382.91
6	3.42	-12.50	6999.52	7293.60	4.20	332.76
7	2.82	13.50	6164.80	6406.72	3.90	380.86
8	2.97	-24.50	6356.64	7011.84	10.30	416.71
9	1.79	28.50	6891.52	6992.64	1.50	408.18
10	2.07	-4.10	6144.32	6232.48	1.40	331.60
11	3.61	-4.80	5516.80	5778.56	4.70	382.67
12	1.44	-24.50	5150.56	5283.52	2.60	291.69
13	1.70	15.90	6535.36	6544.96	.10	311.48
14	5.10	36.40	6492.48	6488.48	-.10	330.44
15	3.92	11.90	5765.28	5673.76	-1.60	354.19
16	2.18	-20.10	6188.00	6622.72	7.00	296.81
17	1.77	-26.90	5729.12	5879.36	2.60	335.12
18	3.46	-13.40	6089.92	6376.00	4.70	329.73
19	3.22	-9.30	6976.80	6939.20	-.50	372.06
20	2.62	43.20	6847.20	6842.56	-.10	368.79
21	1.51	-27.00	6188.32	6226.56	.60	337.35
22	1.87	2.70	6024.48	5950.08	-1.20	323.25
23	3.15	5.50	6034.24	5632.32	-6.70	350.74
24	2.92	5.80	5926.40	5901.60	-.40	365.21
25	2.87	26.70	5622.40	5594.24	-.50	317.27
26	1.57	-7.90	6740.80	6898.56	2.30	446.29
27	3.27	11.10	6193.28	6076.48	-1.90	324.81
28	2.17	-14.10	6478.08	6573.44	1.50	358.46
29	2.29	4.10	7328.96	6985.12	-4.70	353.63
30	3.01	2.40	6477.60	6225.12	-3.90	382.46
31	2.29	-15.80	6266.88	6331.20	1.00	364.75

	Pt66TOTCnt	PctChg66TO	Pre66TOTDe	Pt66TOTDen	PctChg66TO	Pre66TOTAr
1	343.66	.0	861.40	856.40	-.60	399.04
2	388.80	.50	782.10	780.30	-.20	494.72
3	322.40	1.20	806.80	797.70	-1.10	395.04
4	341.22	.60	609.60	613.00	.60	556.64
5	380.83	-.50	713.50	710.30	-.40	536.64
6	333.09	.10	779.20	775.60	-.50	427.04
7	380.54	-.10	788.50	789.40	.10	483.04
8	416.08	-.20	776.30	768.00	-1.10	536.80
9	408.28	.0	822.40	819.70	-.30	496.32
10	330.74	-.30	614.80	616.90	.30	539.36
11	384.84	.60	714.60	707.80	-1.00	535.52
12	295.65	1.40	711.90	667.80	-6.20	409.76
13	313.01	.50	712.80	716.30	.50	436.96
14	338.00	2.30	660.70	664.30	.50	500.16
15	354.08	.0	713.00	709.80	-.40	496.80
16	295.97	-.30	735.60	713.40	-3.00	403.52
17	336.34	.40	788.90	791.50	.30	424.80
18	339.00	2.80	660.10	678.60	2.80	499.52
19	367.92	-1.10	776.10	769.60	-.80	479.36
20	372.56	1.00	751.80	745.60	-.80	490.56
21	336.92	-.10	633.90	627.30	-1.00	532.16
22	323.56	.10	687.00	692.60	.80	470.56
23	345.38	-1.50	788.00	770.70	-2.20	445.12
24	367.68	.70	754.60	748.80	-.80	484.00
25	320.02	.90	739.10	743.80	.60	429.28
26	446.24	.0	723.60	714.40	-1.30	616.80
27	325.27	.10	769.80	768.90	-.10	421.92
28	363.67	1.50	679.10	666.20	-1.90	527.84
29	356.45	.80	647.40	646.90	-.10	546.24
30	381.33	-.30	761.50	761.20	.0	502.24
31	365.33	.20	730.40	722.30	-1.10	499.36

	Pt66TOTAr	PctChg66TO	Pre66CrtCnt	Pt66CrtCnt	PctChg66Crt	Pre66CrtDen
1	401.28	.60	313.93	313.05	-.30	1154.10
2	498.24	.70	360.83	363.02	.60	1183.80
3	404.16	2.30	304.83	309.09	1.40	1169.50
4	556.64	.0	300.52	300.09	-.10	1163.00
5	536.16	-.10	352.20	349.19	-.90	1175.90
6	429.44	.60	307.30	308.58	.40	1173.20
7	482.08	-.20	355.54	353.88	-.50	1192.80
8	541.76	.90	389.96	387.88	-.50	1156.20
9	498.08	.40	378.18	378.31	.0	1170.10
10	536.16	-.60	301.64	302.25	.20	1168.00
11	543.68	1.50	351.60	353.87	.60	1152.90
12	442.72	8.00	276.75	276.43	-.10	1185.50
13	436.96	.0	279.57	281.66	.70	1154.90
14	508.80	1.70	282.44	289.25	2.40	1120.80
15	498.88	.40	323.43	322.62	-.30	1133.70
16	414.88	2.80	275.40	269.35	-2.20	1156.80
17	424.96	.0	311.12	312.62	.50	1158.80
18	499.52	.0	308.63	321.26	4.10	1179.80
19	478.08	-.30	342.84	334.91	-2.30	1134.90
20	499.68	1.90	341.80	342.87	.30	1178.30
21	537.12	.90	307.22	307.77	.20	1173.70
22	467.20	-.70	297.97	298.74	.30	1154.60
23	448.16	.70	332.73	321.96	-3.20	1166.30
24	491.04	1.50	337.47	339.80	.70	1156.30
25	430.24	.20	291.19	295.99	1.60	1148.20
26	624.64	1.30	410.36	407.08	-.80	1179.70
27	423.04	.30	306.82	306.43	-.10	1183.70
28	545.92	3.40	309.73	313.73	1.30	1119.60
29	551.04	.90	326.55	328.93	.70	1148.50
30	500.96	-.30	357.74	355.60	-.60	1143.10
31	505.76	1.30	328.69	328.72	.0	1158.00

	Pt66CrtDen	PctChg66Crt	Pre66CAr	Pt66CAr	PctChg66CAr	Pre66CrtThk
1	1152.90	-.10	272.00	271.52	-.20	4.91
2	1180.50	-.30	304.80	307.52	.90	4.77
3	1171.50	.20	260.64	263.84	1.20	4.67
4	1167.10	.40	258.40	257.12	-.50	3.57
5	1175.90	.0	299.52	296.96	-.90	4.38
6	1165.30	-.70	261.92	264.80	1.10	4.41
7	1196.20	.30	298.08	295.84	-.80	4.73
8	1154.90	-.10	337.28	335.84	-.40	5.10
9	1167.60	-.20	323.20	324.00	.20	5.15
10	1171.20	.30	258.24	258.08	-.10	3.64
11	1147.10	-.50	304.96	308.48	1.20	4.49
12	1181.70	-.30	233.44	233.92	.20	3.93
13	1165.00	.90	242.08	241.76	-.10	3.92
14	1132.00	1.00	252.00	255.52	1.40	3.73
15	1136.00	.20	285.28	284.00	-.40	4.37
16	1156.20	-.10	238.08	232.96	-2.20	4.08
17	1163.00	.40	268.48	268.80	.10	4.57
18	1182.50	.20	261.60	271.68	3.90	3.91
19	1142.60	.70	302.08	293.12	-3.00	4.84
20	1175.50	-.20	290.08	291.68	.60	4.51
21	1169.30	-.40	261.76	263.20	.60	3.74
22	1156.10	.10	258.08	258.40	.10	4.02
23	1135.60	-2.60	285.28	283.52	-.60	4.77
24	1156.70	.0	291.84	293.76	.70	4.59
25	1151.90	.30	253.60	256.96	1.30	4.21
26	1175.20	-.40	347.84	346.40	-.40	4.76
27	1179.30	-.40	259.20	259.84	.20	4.39
28	1111.60	-.70	276.64	282.24	2.00	4.02
29	1153.70	.50	284.32	285.12	.30	4.06
30	1141.50	-.10	312.96	311.52	-.50	4.88
31	1149.70	-.70	283.84	285.92	.70	4.33

	Pt66CrtThk	PctChg66Crt	Pre66PERIC	Pt66PERIC	Pre66ENDOC	Pt66ENDOC
1	4.88	-.70	70.81	71.01	39.96	40.38
2	4.80	.60	78.85	79.13	48.85	48.96
3	4.66	-.30	70.46	71.27	41.10	41.99
4	3.55	-.60	83.64	83.64	61.22	61.35
5	4.34	-1.00	82.12	82.08	54.59	54.83
6	4.45	1.00	73.26	73.46	45.55	45.49
7	4.69	-.80	77.91	77.83	48.21	48.38
8	5.04	-1.30	82.13	82.51	50.07	50.87
9	5.15	.0	78.97	79.11	46.64	46.77
10	3.66	.40	82.33	82.08	59.44	59.11
11	4.50	.30	82.03	82.66	53.83	54.37
12	3.72	-5.30	71.76	74.59	47.07	51.22
13	3.91	-.20	74.10	74.10	49.49	49.53
14	3.75	.50	79.28	79.96	55.84	56.42
15	4.33	-.90	79.01	79.18	51.56	51.96
16	3.88	-4.80	71.21	72.21	45.60	47.81
17	4.58	.10	73.06	73.08	44.32	44.30
18	4.09	4.80	79.23	79.23	54.68	53.51
19	4.66	-3.70	77.61	77.51	47.20	48.21
20	4.48	-.70	78.52	79.24	50.19	51.13
21	3.74	.0	81.78	82.16	58.29	58.67
22	4.04	.70	76.90	76.62	51.67	51.22
23	4.71	-1.40	74.79	75.05	44.82	45.49
24	4.58	-.30	77.99	78.55	49.14	49.79
25	4.28	1.50	73.45	73.53	46.99	46.66
26	4.69	-1.40	88.04	88.60	58.14	59.13
27	4.40	.10	72.82	72.91	45.22	45.29
28	4.02	.0	81.44	82.83	56.18	57.56
29	4.04	-.30	82.85	83.21	57.37	57.81
30	4.86	-.40	79.44	79.34	48.77	48.79
31	4.32	.0	79.22	79.72	52.04	52.56

	Pre38TOTCn	Pt38TOTCnt	PctChg38TO	Pre38TOTDe	Pt38TOTDen	PctChg38TO
1	327.59	329.38	.50	1034.00	1038.70	.50
2	329.54	328.25	-.40	952.20	951.60	-.10
3	242.34	270.69	11.70	771.20	843.00	9.30
4	289.65	289.51	.0	798.20	795.70	-.30
5	330.73	332.69	.60	884.90	888.60	.40
6	297.38	298.88	.50	948.30	951.10	.30
7	350.56	348.12	-.70	949.30	947.20	-.20
8	385.90	390.65	1.20	928.70	933.30	.50
9	356.90	355.61	-.40	981.80	980.80	-.10
10	296.47	296.16	-.10	912.80	911.40	-.20
11	319.65	324.14	1.40	887.10	895.60	1.00
12	238.85	250.94	5.10	889.10	903.40	1.60
13	280.86	286.87	2.10	963.90	943.10	-2.20
14	284.72	287.56	1.00	836.20	836.30	.0
15	320.90	319.20	-.50	907.10	905.60	-.20
16	258.04	262.10	1.60	971.00	978.60	.80
17	279.97	281.21	.40	918.50	922.10	.40
18	293.28	289.51	-1.30	867.90	863.30	-.50
19	320.50	323.36	.90	991.60	991.20	.0
20	308.99	305.96	-1.00	902.40	921.10	2.10
21	309.03	310.11	.30	895.00	896.10	.10
22	290.25	292.12	.60	934.10	933.90	.0
23	309.37	305.73	-1.20	986.00	978.40	-.80
24	303.39	305.52	.70	898.20	900.70	.30
25	285.08	282.58	-.90	897.20	899.20	.20
26	366.62	368.42	.50	907.80	908.00	.0
27	273.61	275.29	.60	944.80	940.20	-.50
28	329.97	335.46	1.70	924.40	942.70	2.00
29	322.37	326.23	1.20	905.90	918.00	1.30
30	321.24	323.21	.60	876.80	878.30	.20
31	323.92	325.85	.60	963.60	957.00	-.70

	Pre38TOTAr	Pt38TOTAr	PctChg38TO	Pre38CrtCnt	Pt38CrtCnt	PctChgCrtCn
1	316.80	317.12	.10	317.66	319.70	.60
2	346.08	344.96	-.30	319.12	319.05	.0
3	314.24	321.12	2.20	231.35	261.03	12.80
4	362.88	363.84	.30	278.54	277.65	-.30
5	373.76	374.40	.20	318.60	319.86	.40
6	313.60	314.24	.20	283.36	284.67	.50
7	369.28	367.52	-.50	336.34	333.62	-.80
8	415.52	418.56	.70	365.84	368.16	.60
9	363.52	362.56	-.30	342.75	342.18	-.20
10	324.80	324.96	.0	284.74	283.93	-.30
11	360.32	361.92	.40	306.60	308.63	.70
12	268.64	277.76	3.40	230.32	242.40	5.20
13	291.36	304.16	4.40	267.94	273.57	2.10
14	340.48	343.84	1.00	256.51	259.72	1.30
15	353.76	352.48	-.40	307.93	306.87	-.30
16	265.76	267.84	.80	249.42	254.06	1.90
17	304.80	304.96	.10	269.13	270.65	.60
18	337.92	335.36	-.80	280.35	276.18	-1.50
19	323.20	326.24	.90	305.02	307.25	.70
20	342.40	332.16	-3.00	294.66	294.00	-.20
21	345.28	346.08	.20	291.91	292.16	.10
22	310.72	312.80	.70	280.93	282.81	.70
23	313.76	312.48	-.40	302.27	297.22	-1.70
24	337.76	339.20	.40	287.86	288.96	.40
25	317.76	314.24	-1.10	276.25	273.59	-1.00
26	403.84	405.76	.50	357.08	358.00	.30
27	289.60	292.80	1.10	261.96	263.01	.40
28	356.96	355.84	-.30	308.02	315.84	2.50
29	355.84	355.36	-.10	312.56	316.93	1.40
30	366.40	368.00	.40	306.52	308.70	.70
31	336.16	340.48	1.30	310.77	312.01	.40

	Pre38CrtDen	Pt38CrtDen	PctChg38Crt	Pre38CAr	Pt38CAr	PctChgCAr
1	1174.80	1180.20	.50	270.40	270.88	.20
2	1221.40	1219.60	-.10	261.28	261.60	.10
3	1185.20	1187.40	.20	195.20	219.84	12.60
4	1218.20	1213.50	-.40	228.64	228.80	.10
5	1211.90	1212.30	.0	262.88	263.84	.40
6	1195.00	1194.10	-.10	237.12	238.40	.50
7	1218.60	1219.40	.10	276.00	273.60	-.90
8	1161.90	1162.10	.0	314.88	316.80	.60
9	1190.80	1190.80	.0	287.84	287.36	-.20
10	1195.20	1194.20	-.10	238.24	237.76	-.20
11	1165.60	1171.20	.50	263.04	263.52	.20
12	1222.00	1221.80	.0	188.48	198.40	5.30
13	1198.70	1167.90	-2.60	223.52	234.24	4.80
14	1168.50	1168.70	.0	219.52	222.24	1.20
15	1161.50	1164.50	.30	265.12	263.52	-.60
16	1193.60	1192.10	-.10	208.96	213.12	2.00
17	1193.80	1197.10	.30	225.44	226.08	.30
18	1187.90	1190.40	.20	236.00	232.00	-1.70
19	1164.50	1165.90	.10	261.92	263.52	.60
20	1174.50	1210.50	3.10	250.88	242.88	-3.20
21	1187.00	1187.30	.0	245.92	246.08	.10
22	1188.80	1189.50	.10	236.32	237.76	.60
23	1190.40	1183.20	-.60	253.92	251.20	-1.10
24	1175.90	1177.30	.10	244.80	245.44	.30
25	1187.40	1193.20	.50	232.64	229.28	-1.40
26	1210.90	1208.80	-.20	294.88	296.16	.40
27	1203.00	1194.60	-.70	217.76	220.16	1.10
28	1137.80	1160.50	2.00	270.72	272.16	.50
29	1178.20	1191.10	1.10	265.28	266.08	.30
30	1173.90	1175.00	.10	261.12	262.72	.60
31	1210.90	1202.30	-.70	256.64	259.52	1.10

	Pre38CrtThk	Pt38CrtThk	PctChg38Crt	Pre38PERIC	Pt38PERIC	Pre38ENDOC
1	6.20	6.21	.20	63.10	63.13	24.15
2	5.30	5.33	.50	65.95	65.84	32.64
3	3.85	4.43	15.20	62.84	63.52	38.68
4	4.21	4.21	-.10	67.53	67.62	41.07
5	4.97	4.98	.30	68.53	68.59	37.33
6	5.06	5.09	.60	62.78	62.84	31.00
7	5.39	5.35	-.80	68.12	67.96	34.24
8	5.84	5.85	.20	72.26	72.52	35.56
9	5.85	5.85	.0	67.59	67.50	30.84
10	4.92	4.90	-.30	63.89	63.90	32.98
11	5.15	5.14	-.20	67.29	67.44	34.96
12	4.20	4.38	4.30	58.10	59.08	31.74
13	4.98	5.12	2.80	60.51	61.82	29.20
14	4.21	4.24	.80	65.41	65.73	38.99
15	5.30	5.27	-.50	66.67	66.55	33.38
16	4.95	5.06	2.30	57.79	58.02	26.72
17	4.82	4.84	.40	61.89	61.91	31.58
18	4.68	4.60	-1.70	65.17	64.92	35.79
19	5.73	5.72	-.10	63.73	64.03	27.75
20	5.04	4.95	-1.80	65.60	64.61	33.91
21	4.86	4.85	-.10	65.87	65.95	35.34
22	5.08	5.09	.20	62.49	62.70	30.58
23	5.63	5.56	-1.30	62.79	62.66	27.42
24	4.93	4.93	.0	65.15	65.29	34.18
25	4.85	4.80	-1.10	63.19	62.84	32.71
26	5.45	5.46	.20	71.24	71.41	37.00
27	4.82	4.85	.60	60.33	60.66	30.05
28	5.42	5.48	1.10	66.98	66.87	32.92
29	5.27	5.31	.60	66.87	66.83	33.73
30	5.01	5.03	.50	67.86	68.00	36.37
31	5.31	5.33	.40	65.00	65.41	31.61

	Pt38ENDOC	Pre4TotCnt	Pt4TotCnt	PctChg4TotC	Pre4TotDen	Pt4TotDen
1	24.11	322.81	319.20	-1.10	351.10	348.60
2	32.37	402.03	372.02	-7.50	321.20	307.00
3	35.68	229.46	297.94	29.80	300.50	251.30
4	41.19	406.75	410.91	1.00	328.20	326.70
5	37.27	297.25	280.28	-5.70	250.30	242.10
6	30.87	308.79	317.34	2.80	301.90	309.50
7	34.36	516.38	516.01	-.10	439.60	441.60
8	35.76	375.52	312.50	-16.80	285.90	249.80
9	30.74	479.27	472.74	-1.40	340.40	332.50
10	33.10	302.94	305.89	1.00	245.80	245.80
11	35.16	318.81	303.81	-4.70	282.90	285.90
12	31.58	433.84	269.71	-37.80	454.80	321.30
13	29.64	305.09	318.97	4.50	291.50	298.20
14	39.09	342.29	325.55	-4.90	296.10	295.90
15	33.44	273.24	262.82	-3.80	264.10	267.10
16	26.22	300.08	272.03	-9.30	254.70	248.20
17	31.48	284.79	280.48	-1.50	278.00	277.00
18	36.04	386.85	423.50	9.50	290.20	322.00
19	28.07	318.55	321.49	.90	299.70	299.50
20	33.50	436.32	410.56	-5.90	392.10	371.60
21	35.45	280.71	283.09	.80	258.20	266.80
22	30.71	321.70	335.59	4.30	291.30	299.50
23	27.75	419.02	365.21	-12.80	369.20	329.90
24	34.33	368.47	300.67	-18.40	298.40	251.90
25	32.68	265.98	270.94	1.90	316.10	318.20
26	37.11	429.68	416.54	-3.10	329.50	322.00
27	30.21	312.11	313.76	.50	260.00	264.90
28	32.43	447.67	389.12	-13.10	358.00	322.50
29	33.50	290.88	287.87	-1.00	319.70	323.40
30	36.37	322.78	321.62	-.40	272.70	271.90
31	31.90	314.88	314.61	-.10	338.70	337.30

	PctChg4TotL	Pre4TrCnt	Pt4TrCnt	PctChg4TrCn	Pre4TrDen	Pt4TrDen
1	-.70	253.23	241.38	-4.70	315.50	306.40
2	-4.40	343.80	317.55	-7.60	308.10	292.50
3	-16.40	195.38	258.42	32.30	302.90	245.10
4	-.50	349.17	354.04	1.40	319.60	319.30
5	-3.30	231.31	215.72	-6.70	220.00	210.70
6	2.50	233.75	235.66	.80	260.90	264.90
7	.50	357.34	354.63	-.80	388.90	389.70
8	-12.60	314.71	253.13	-19.60	268.20	226.40
9	-2.30	399.80	392.71	-1.80	318.20	309.70
10	.0	229.78	233.07	1.40	210.30	211.40
11	1.10	249.38	229.18	-8.10	250.10	246.40
12	-29.40	301.94	205.32	-32.00	407.90	281.20
13	2.30	245.49	255.77	4.20	264.20	269.70
14	-.10	284.07	268.08	-5.60	275.70	276.70
15	1.10	206.23	195.51	-5.20	227.80	226.90
16	-2.60	242.55	210.44	-13.20	230.60	216.00
17	-.40	223.12	220.21	-1.30	245.90	245.40
18	11.00	325.13	348.65	7.20	272.80	302.60
19	-.10	259.77	262.62	1.10	275.50	276.30
20	-5.20	346.46	337.20	-2.70	361.40	346.90
21	3.30	210.59	209.55	-.50	219.90	225.10
22	2.80	255.19	263.64	3.30	261.40	267.60
23	-10.60	331.35	275.44	-16.90	336.30	287.80
24	-15.60	310.21	244.08	-21.30	281.60	229.00
25	.70	199.14	203.55	2.20	273.00	275.10
26	-2.30	365.89	349.90	-4.40	314.90	303.20
27	1.90	248.63	253.82	2.10	233.60	241.50
28	-9.90	375.36	314.17	-16.30	338.00	294.20
29	1.20	216.52	210.94	-2.60	275.30	275.80
30	-.30	259.96	261.20	.50	247.50	248.30
31	-.40	232.67	231.67	-.40	291.80	290.20

	PctChg4TrDe	Pre4TOTAr	Pt4TOTAr	PctChg4TOT	Pre4TrAr	Pt4TrAr
1	-2.90	919.36	915.52	-.40	802.56	787.68
2	-5.10	1251.68	1211.68	-3.20	1115.68	1085.76
3	-19.10	763.68	1185.44	55.20	645.12	1054.56
4	-.10	1239.20	1257.60	1.50	1092.48	1108.64
5	-4.20	1187.52	1157.92	-2.50	1051.52	1023.84
6	1.50	1022.88	1025.28	.20	895.84	889.60
7	.20	1174.56	1168.48	-.50	918.88	909.92
8	-15.60	1313.60	1251.04	-4.80	1173.44	1118.24
9	-2.70	1408.00	1421.60	1.00	1256.48	1268.00
10	.50	1232.64	1244.64	1.00	1092.80	1102.24
11	-1.50	1127.04	1062.72	-5.70	997.12	930.24
12	-31.10	953.92	839.52	-12.00	740.32	730.24
13	2.10	1046.56	1069.60	2.20	929.12	948.32
14	.40	1155.84	1100.32	-4.80	1030.24	968.80
15	-.40	1034.72	984.00	-4.90	905.44	861.76
16	-6.30	1178.24	1095.84	-7.00	1052.00	974.08
17	-.20	1024.48	1012.64	-1.20	907.36	897.28
18	10.90	1332.96	1315.36	-1.30	1191.84	1152.16
19	.30	1062.88	1073.60	1.00	943.04	950.40
20	-4.00	1112.80	1104.80	-.70	958.56	972.00
21	2.40	1087.04	1060.96	-2.40	957.60	931.04
22	2.40	1104.48	1120.64	1.50	976.32	985.12
23	-14.40	1135.04	1107.04	-2.50	985.28	957.12
24	-18.70	1234.72	1193.60	-3.30	1101.60	1066.08
25	.80	841.44	851.52	1.20	729.44	739.84
26	-3.70	1304.16	1293.76	-.80	1162.08	1154.08
27	3.40	1200.32	1184.64	-1.30	1064.48	1051.20
28	-13.00	1250.56	1206.40	-3.50	1110.56	1068.00
29	.20	909.92	890.08	-2.20	786.56	764.96
30	.30	1183.68	1182.88	-.10	1050.24	1052.00
31	-.50	929.76	932.80	.30	797.44	798.40

	PctChg4TrAr	PreFemTCNT	PtFemTCNT	PreFemTDEN	PtFemTDEN	PreFemTAr
1	-1.90	437.78	443.90	989.20	982.40	442.56
2	-2.70	443.27	443.83	851.90	853.50	520.32
3	63.50	317.68	317.62	785.70	796.90	404.32
4	1.50	404.80	407.97	738.50	739.90	548.16
5	-2.60	422.15	423.48	840.00	841.60	502.56
6	-.70	398.95	402.24	952.80	964.70	418.72
7	-1.00	446.68	445.54	840.90	842.80	531.20
8	-4.70	473.27	470.53	949.90	950.80	498.24
9	.90	510.98	513.86	962.20	961.60	531.04
10	.90	384.60	384.91	794.90	797.90	483.84
11	-6.70	438.83	438.18	802.00	805.70	547.20
12	-1.40	304.38	308.18	814.70	819.30	373.60
13	2.10	376.11	370.99	884.40	876.30	425.28
14	-6.00	407.95	411.56	765.70	767.10	532.80
15	-4.80	428.85	432.06	901.20	905.30	475.84
16	-7.40	350.58	351.09	842.70	844.60	416.00
17	-1.10	398.41	395.08	919.90	924.50	433.12
18	-3.30	389.84	396.78	771.00	780.10	505.60
19	.80	422.88	422.86	958.30	959.00	441.28
20	1.40	446.04	442.43	928.60	926.10	480.32
21	-2.80	390.30	385.84	828.90	808.20	470.88
22	.90	399.48	402.24	860.60	862.10	464.16
23	-2.90	413.61	414.53	882.00	868.80	468.96
24	-3.20	440.78	439.66	898.50	888.70	490.56
25	1.40	365.11	367.78	885.50	881.40	412.32
26	-.70	457.80	456.71	815.90	823.10	561.12
27	-1.20	387.60	389.93	897.20	897.60	432.00
28	-3.80	449.46	455.72	922.20	926.60	487.36
29	-2.70	429.16	430.62	815.30	838.70	526.40
30	.20	387.53	386.05	793.30	790.60	488.48
31	.10	494.12	487.21	954.10	940.10	517.92

	PtFemTAr	PreFemCCNT	PtFemCCNT	PreFemCDEN	PtFemCDEN	PreFemCAr
1	451.84	422.97	425.84	1125.90	1120.20	375.68
2	520.00	422.27	422.82	1152.00	1156.00	366.56
3	398.56	298.52	299.74	1118.60	1126.50	266.88
4	551.36	382.94	385.76	1147.90	1154.70	333.60
5	503.20	403.54	406.76	1163.30	1166.20	346.88
6	416.96	384.81	389.01	1141.50	1157.80	337.12
7	528.64	425.13	427.30	1168.50	1175.40	363.84
8	494.88	460.48	457.62	1157.70	1158.40	397.76
9	534.40	490.34	493.96	1153.00	1152.00	425.28
10	482.40	369.48	368.18	1169.30	1177.00	316.00
11	543.84	420.34	419.37	1114.60	1117.20	377.12
12	376.16	290.68	295.12	1135.50	1158.60	256.00
13	423.36	363.55	357.33	1148.10	1145.30	316.64
14	536.48	368.70	367.41	1077.80	1084.70	342.08
15	477.28	412.63	417.36	1141.10	1143.60	361.60
16	415.68	333.79	335.81	1128.90	1130.80	295.68
17	427.36	383.02	378.12	1150.90	1153.90	332.80
18	508.64	372.41	378.49	1155.70	1155.60	322.24
19	440.96	408.99	408.63	1151.40	1155.60	355.20
20	477.76	429.66	425.72	1161.00	1162.40	370.08
21	477.44	372.55	366.98	1166.00	1158.40	319.52
22	466.56	383.96	389.41	1151.50	1156.20	333.44
23	477.12	400.51	395.92	1143.00	1120.20	350.40
24	494.72	419.47	412.43	1131.50	1108.70	370.72
25	417.28	349.35	352.41	1136.60	1131.90	307.36
26	554.88	435.87	435.79	1163.20	1173.00	374.72
27	434.40	374.09	374.34	1151.80	1156.50	324.80
28	491.84	427.05	437.38	1115.40	1113.90	382.88
29	513.44	404.95	407.38	1132.90	1163.70	357.44
30	488.32	367.46	363.64	1142.60	1133.60	321.60
31	518.24	477.67	472.14	1152.70	1152.70	414.40

	PtFemCAr	PreFemCThk	PtFemCThk	PreFemMCS	PtFemMCSA	PctChgMCSA
1	380.16	7.26	7.22	11666.08	11083.20	-5.00
2	365.76	5.87	5.86	9595.04	9374.72	-2.30
3	266.08	4.73	4.77	10062.08	9759.36	-3.00
4	334.08	4.95	4.93	11520.16	12347.20	7.20
5	348.80	5.61	5.65	9773.28	9734.88	-.40
6	336.00	6.45	6.44	12084.80	11560.16	-4.30
7	363.52	5.71	5.72	10492.16	10762.40	2.60
8	395.04	6.94	6.91	11061.44	12105.44	9.40
9	428.80	7.20	7.25	13096.00	12822.24	-2.10
10	312.80	5.10	5.04	9950.40	9743.36	-2.10
11	375.36	5.84	5.83	11847.52	11874.88	.20
12	254.72	4.79	4.73	7870.40	8081.76	2.70
13	312.00	5.75	5.66	11002.88	10844.48	-1.40
14	338.72	5.23	5.13	10536.80	10323.20	-2.00
15	364.96	6.28	6.35	11296.64	11026.08	-2.40
16	296.96	5.32	5.36	9095.36	9608.96	5.60
17	327.68	6.09	6.03	9516.96	9862.08	3.60
18	327.52	5.05	5.13	11330.72	11063.20	-2.40
19	353.60	6.62	6.57	11603.52	11466.08	-1.20
20	366.24	6.44	6.37	12698.24	12451.52	-1.90
21	316.80	5.30	5.18	11268.16	10843.84	-3.80
22	336.80	5.71	5.76	10497.12	9808.64	-6.60
23	353.44	6.08	6.05	12237.76	10440.80	-14.70
24	372.00	6.32	6.30	10257.60	9602.08	-6.40
25	311.36	5.68	5.72	10256.32	9923.36	-3.20
26	371.52	5.66	5.65	13038.88	13250.72	1.60
27	323.68	5.89	5.82	9490.72	9270.72	-2.30
28	392.64	6.69	6.89	12272.96	12374.72	.80
29	350.08	5.61	5.57	11124.64	11554.72	3.90
30	320.80	5.18	5.17	10113.12	10170.88	.60
31	409.60	7.10	6.96	10534.08	10470.72	-.60

	PreTBbmd	PtTBbmd	PrefnTscr	PrefnZscr	PreTrcTscr	PreTrcZscr
1	1.077	1.075	.	.	.	.
2	1.230	1.212	.2	-.1	-.2	-.4
3	.994	.998	-1.0	-.9	-1.7	-1.5
4	1.120	1.068	-.9	-2.1	-1.9	-2.8
5	1.077	1.078	-.7	-.5	-.7	-.5
6	1.045	1.060	.	.	.	.
7	1.147	1.161	.2	.0	-.5	-.7
8	1.188	1.184	.6	.5	.7	.6
9	1.213	1.226	1.2	1.1	2.1	2.0
10	1.081	1.095	-.9	-.9	-1.7	-1.6
11	1.060	1.060	.	.	.	.
12	1.066	1.057	-.7	-.3	-1.0	-.5
13	1.124	1.120	-.7	-.6	-.5	-.3
14	1.048	1.046	.1	.1	-.7	-.7
15	1.149	1.135	-.5	-.4	-.3	.0
16	.961	.954	.	.	.	.
17	1.114	1.099	-1.0	-.8	-.6	-.3
18	1.058	1.060	-.4	-.4	-1.6	-1.6
19	1.063	1.061	.	.	.	.
20	1.095	1.207	.	.	.	.
21	.966	.979	.	.	.	.
22	1.102	1.109	-.3	-.1	-1.0	-.8
23	1.120	1.126	.5	.6	.3	.4
24	1.080	1.066	.	.	.	.
25	1.156	1.146	.3	.4	.3	.6
26	1.305	1.313	.4	-.7	-.1	-.9
27	1.153	1.133	-.6	-.4	-.8	-.6
28	1.221	1.222	.4	.2	.9	.8
29	1.219	1.225	.8	.8	.0	.1
30	1.016	1.057	.	.	.	.
31	1.259	1.255	1.4	1.4	.3	.3

	PreThTscr	PreThZscr	PreSpTscr	PreSpZscr	Pre38SSI	Pt38SSI
1	.	.	.	.6	1314.73	1330.44
2	.6	.4	-.5	-.7	1488.50	1472.32
3	-1.0	-.7	-1.1	-.7	1194.91	1221.85
4	-1.3	-2.5	-1.6	-2.6	1491.65	1477.02
5	-.6	-.5	-.7	-.7	1644.70	1659.15
6	.	.	.	1.2	1282.15	1264.24
7	.2	.0	-.4	-.6	1611.44	1620.73
8	1.4	1.4	.2	.3	1925.13	1943.99
9	1.7	1.6	.8	.8	1549.82	1566.71
10	-1.2	-1.1	-.2	.0	1300.04	1286.76
11	.	.	.	1.5	1509.14	1537.02
12	-.3	.1	-1.5	-.6	995.62	1070.22
13	-.4	-.2	.3	.7	1102.67	1176.42
14	-.2	-.2	-1.1	-1.0	1339.59	1363.93
15	.3	.6	-.6	-.2	1468.87	1447.51
16	.	.	.	.4	1015.02	1029.81
17	-.4	-.2	-.5	-.1	1192.94	1196.92
18	-1.1	-1.0	-.9	-.9	1380.88	1407.28
19	.	.	.	1.4	1284.05	1302.96
20	.	.	.	.4	1403.17	1386.82
21	.	.	.	-.8	1413.08	1401.68
22	-.5	-.2	.1	.4	1220.45	1255.26
23	.5	.6	.0	.3	1242.19	1277.04
24	.	.	.	.4	1367.82	1374.04
25	.8	1.0	.5	.9	1270.11	1245.14
26	.8	-.3	1.7	.9	1839.82	1845.75
27	-.2	.0	1.1	1.5	1116.73	1168.26
28	1.4	1.3	1.7	1.6	1490.22	1500.65
29	.6	.6	1.5	1.6	1509.28	1516.33
30	.	.	.	-1.4	1518.46	1505.76
31	1.5	1.5	2.4	2.5	1459.25	1477.70

	PctChg38SSI	Pre66SSI	Pt66SSI	PctChg66SSI	PctChg66Per	PctChg66Enc
1	1.2	1658.10	1675.79	1.1	.30	1.10
2	-1.1	2255.34	2235.48	-.9	.40	.20
3	2.3	1615.29	1605.80	-.6	1.10	2.20
4	-1.0	2329.24	2330.38	.0	.0	.20
5	.9	2469.03	2476.35	.3	.0	.40
6	-1.4	1859.34	1847.51	-.6	.30	-.10
7	.6	2255.03	2283.13	1.2	-.10	.30
8	1.0	2511.42	2558.76	1.9	.50	1.60
9	1.1	2290.84	2324.43	1.5	.20	.30
10	-1.0	2125.76	2150.60	1.2	-.30	-.50
11	1.8	2368.70	2417.58	2.1	.80	1.00
12	7.5	1662.30	1803.12	8.5	3.90	8.80
13	6.7	1800.56	1850.41	2.8	.0	.10
14	1.8	1953.21	2000.49	2.4	.90	1.00
15	-1.5	2108.83	2098.28	-.5	.20	.80
16	1.5	1647.23	1646.82	.0	1.40	4.90
17	.3	1773.72	1777.02	.2	.0	.0
18	1.9	2175.45	2205.75	1.4	.0	-2.10
19	1.5	2144.54	2079.74	-3.0	-.10	2.10
20	-1.2	2139.74	2220.79	3.8	.90	1.90
21	-.8	2214.72	2225.85	.5	.50	.60
22	2.9	1920.95	1938.41	.9	-.40	-.90
23	2.8	1978.49	1905.25	-3.7	.30	1.50
24	.5	2146.28	2150.21	.2	.70	1.30
25	-2.0	1773.83	1767.26	-.4	.10	-.70
26	.3	3007.91	3067.87	2.0	.60	1.70
27	4.6	1811.85	1803.69	-.5	.10	.10
28	.7	2171.58	2244.98	3.4	1.70	2.50
29	.5	2341.79	2403.21	2.6	.40	.80
30	-.8	2207.84	2185.74	-1.0	-.10	.0
31	1.3	2267.41	2156.13	-4.9	.60	1.00

	PctChg38Per	PctChg38End
1	.10	-.20
2	-.20	-.90
3	1.10	-7.80
4	.10	.30
5	.10	-.10
6	.10	-.40
7	-.20	.30
8	.40	.60
9	-.10	-.30
10	.0	.40
11	.20	.60
12	1.70	-.50
13	2.20	1.50
14	.50	.30
15	-.20	.20
16	.40	-1.80
17	.0	-.30
18	-.40	.70
19	.50	1.20
20	-1.50	-1.20
21	.10	.30
22	.30	.40
23	-.20	1.20
24	.20	.40
25	-.60	-.10
26	.20	.30
27	.60	.60
28	-.20	-1.50
29	-.10	-.70
30	.20	.0
31	.60	.90